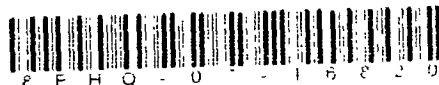




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October 12, 2007



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Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

Re: Phenol, 4,4'-(-1-methylethylidene)bis-, CASRN 80-05-7
Follow-up information regarding 8EHQ-07-16820

Dear Sir or Madam:

The following information is being submitted on behalf of the Polycarbonate/BPA Global Group of the American Chemistry Council. The Polycarbonate/BPA Global Group includes the following companies that manufacture bisphenol A in the US: Bayer MaterialScience, The Dow Chemical Company, SABIC Innovative Plastics (formerly a part of General Electric Company), and Sunoco Inc.

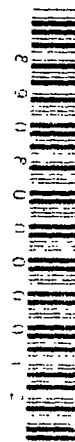
The attached TSCA 8(e) submission in regard to the effects of bisphenol A on terrestrial plants (8EHQ-07-16820) was submitted on April 23, 2007 when information from a study first became available. Enclosed with this letter is the final report from that study titled:

Bisphenol A - Determination of Effects on Seedling Emergence and Seedling Growth (Limit/Range-Finding Tests)

Also enclosed with this letter is the final report from a definitive follow-up study titled:

Bisphenol A - Determination of Effects on Seedling Emergence and Seedling Growth (Definitive Tests)

Beyond the preliminary information reported earlier, the definitive study establishes EC25, EC50, LOEC and NOEC values for each of the plant species in the study. These values should be reviewed in light of other information showing actual or expected levels of bisphenol A in soil. As summarized in our attached April 23, 2007 submission, that



307468



TSCA Section 8(e) Coordinator
Page 2
October 12, 2007

values should be reviewed in light of other information showing actual or expected levels of bisphenol A in soil. As summarized in our attached April 23, 2007 submission, that information shows levels several orders of magnitude lower than the no effect levels determined in the enclosed definitive study.

Both studies were conducted according to OECD Guideline 208: Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test.

If you have any questions, please feel free to contact me on (703) 741-5588 or by e-mail at steve_hentges@americanchemistry.com.

Sincerely,

A handwritten signature in black ink, appearing to read 'S G Hentges', with a stylized flourish at the end.

Steven G. Hentges
Executive Director
Polycarbonate/BPA Global Group
American Chemistry Council



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON D.C. 20460

ML-35169

STEVEN G. HENTGES
POLYCARBONATE/BPA GLOBAL GROUP OF THE
AMERICAN CHEMISTRY COUNCIL
1300 WILSON BOULEVARD
ARLINGTON
VA 22209

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

8e Submission(s)

Dear STEVEN G. HENTGES:

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA), dated 04/30/2007.

Please cite the assigned 8EHQ number(s) listed below when inquiring about the submission(s) or when submitting follow-up information. Address any further correspondence related to the submission(s) to:

Document Control Office (7407W)
U.S. Environmental Protection Agency
ATTN: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460

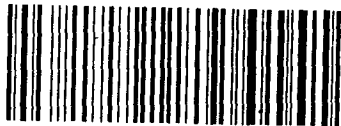
8EHQ Number
8EHQ-07-16820

Chemical
Phenol, 4,4'-(1-methylethylidene)bis-*

07-16820

07-16820

CERTIFIED MAIL



7003 1010 0000 3858 8143



1300 Wilson Boulevard, Arlington VA 22209

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Attn: TSCA Section 8(e) Coordinator
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CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION

April 23, 2007

Document Processing Center (7407M)
(Attn: TSCA Section 8(e) Coordinator)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

Re: Phenol, 4,4'-(1-methylethylidene)bis-, CASRN 80-05-7

Dear Sir or Madam:

The following information is being submitted on behalf of the Polycarbonate/BPA Global Group of the American Chemistry Council. The Polycarbonate/BPA Global Group includes the following companies that manufacture bisphenol A in the US: Bayer Corporation, The Dow Chemical Company, General Electric Company, and Sunoco Inc.

This information is provided pursuant to current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substances Control Act. No determination has been made as to whether a significant risk of injury to health or the environment is actually presented by the findings.

The effects of bisphenol A (BPA) on terrestrial plants was examined according to OECD Guideline 208: Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test. A limit test was performed in accordance with the guidelines.

The test substance was mixed with soil at 150 and 1000 mg/kg. Seeds from six plant varieties were introduced into the soil and monitored for emergence, dry weight biomass per shoot and percent biomass after 28 days relative to controls. The results are attached.

The results of this limit test, showing effects at 150 mg/kg and 1000 mg/kg, should be reviewed in light of other information showing actual or expected levels of BPA in soil. That information shows levels several orders of magnitude lower than the levels tested. For example:

- The UK Environment Agency is conducting an extensive environmental risk



**Summary of Percent Emergence and Seedling Dry Weight Biomass at
Test Termination (Day 21) for the Exposure of Six Plant Species to Bisphenol A**

Cabbage:

Nominal Concentration (mg BPA/kg)	Mean Percent Emergence (%)	Mean Dry Weight Biomass per Shoot (gram)	Percent Reduction of Biomass Relative to Pooled Control (%)
Control	48	0.2984	NA
Solvent Control	70	0.2740	NA
Pooled Control	NC	0.2855	NA
150	30	0.2818	1
1000	0	0.0000	100

Corn:

Nominal Concentration (mg BPA/kg)	Mean Percent Emergence (%)	Mean Dry Weight Biomass per Shoot (gram)	Percent Reduction of Biomass Relative to Pooled Control (%)
Control	100	1.3601	NA
Solvent Control	100	1.3094	NA
Pooled Control	100	1.3348	NA
150	95	0.7242	46
1000	90	0.0265	98

Oat:

Nominal Concentration (mg BPA/kg)	Mean Percent Emergence (%)	Mean Dry Weight Biomass per Shoot (gram)	Percent Reduction of Biomass Relative to Pooled Control (%)
Control	84	0.2106	NA
Solvent Control	94	0.1766	NA
Pooled Control	89	0.2038	NA
150	78	0.1487	27
1000	70	0.0092	95

Soybean:

Nominal Concentration (mg BPA/kg)	Mean Percent Emergence (%)	Mean Dry Weight Biomass per Shoot (gram)	Percent Reduction of Biomass Relative to Pooled Control (%)
Control	100	1.1321	NA
Solvent Control	95	1.0970	NA
Pooled Control	98	1.1145	NA
150	100	1.0132	9
1000	90	0.2293	79

Tomato:

Nominal Concentration (mg BPA/kg)	Mean Percent Emergence (%)	Mean Dry Weight Biomass per Shoot (gram)	Percent Reduction of Biomass Relative to Solvent Control (%)
Control	85	0.7933	NA
Solvent Control	80	0.5226	NA
Pooled Control	83	NC	NC
150	30	0.1681	68
1000	5	0.0005	100

Wheat:

Nominal Concentration (mg BPA/kg)	Mean Percent Emergence (%)	Mean Dry Weight Biomass per Shoot (gram)	Percent Reduction of Biomass Relative to Control (%)
Control	93	0.1671	NA
Solvent Control	70	0.1463	NA
Pooled Control	81	0.1567	NA
150	95	0.0784	50
1000	63	0.0035	98

Notes: NA =Not applicable
NC =Not calculated

Study Title

Bisphenol A - Determination of Effects on
Seedling Emergence and Seedling Growth
(Limit/Range-Finding Tests)

Data Requirement

OECD Guideline Number 208

Author

James R. Hoberg

Study Completed On

23 August 2007

Submitted to

American Chemistry Council
1300 Wilson Boulevard
Arlington, Virginia 22209

Performing Laboratory

Springborn Smithers Laboratories
790 Main Street
Wareham, Massachusetts 02571-1037

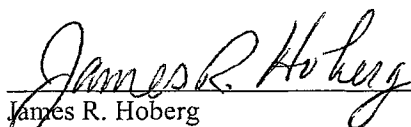
Laboratory Project ID

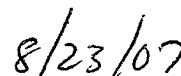
Springborn Smithers Study No. 13761.6123

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for "**Bisphenol A – Determination of Effects on Seedling Emergence and Seedling Growth**" were produced and compiled in accordance with all pertinent OECD Good Laboratory Practice Regulations (OECD, 1998) with the following exceptions: routine soil and water screening analyses were conducted at Agvise Laboratories, Northwood, North Dakota, and GeoLabs, Inc., Braintree, Massachusetts, respectively, using standard U.S. EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

SPRINGBORN SMITHERS LABORATORIES


James R. Hoberg
Study Director


Date

QUALITY ASSURANCE UNIT STATEMENT

The study conduct, raw data and report for "**Bisphenol A – Determination of Effects on Seedling Emergence and Seedling Growth**" were inspected by the Quality Assurance Unit at Springborn Smithers Laboratories to determine adherence with the study protocol and laboratory standard operating procedures. Dates of study inspections, inspection types, and dates reported to the Study Director and to Management are given below.

Inspection Date	Inspection Type	Reported to Study Director/Management
2/14/07	Protocol review	2/14/07
2/19/07	Planting – inlife inspection	2/19/07
4/9/07	Data audit	4/9/07
4/12/07	Data audit	4/13/07
4/13/07	Summary report	4/13/07
7/18/07	Draft report	7/18/07
8/15/07	Revised draft report	8/15/07
8/22/07	Final report	8/22/07

SPRINGBORN SMITHERS LABORATORIES

Kathleen F. Terrio
Kathleen F. Terrio
Quality Assurance Auditor

22 August 2007
Date

KEY STUDY PERSONNEL

The following Springborn Smithers personnel were responsible for the conduct of the work and reporting of the study results.

James R. Hoberg	Study Director
Jeffrey Martin	Biologist I
Andrea Dalton	Assistant Biologist
Jennifer Donovan	Biology Technician II
Kathleen L. Maguire	Assistant Biologist
Donald Gries	Assistant Chemist
Marjorie E. Dix	Senior Research Chemist
Andrea M. Soares	Technical Report Writer
Susan P. Shepherd	Director, Ecotoxicology

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SUMMARY

Bisphenol A - Determination of Effects on Seedling Emergence and Seedling Growth

SPONSOR: American Chemistry Council

PROTOCOL TITLE: Seedling Emergence and Seedling Growth Test Following OECD Draft Guideline #208", Springborn Smithers Laboratories Protocol No.:101006/OECD/Emergence and Growth/6 species/BPA

SPRINGBORN SMITHERS STUDY NUMBER: 13761.6123

TEST SUBSTANCES: Stock solutions containing mixtures of [¹⁴C]bisphenol A and [¹²C]bisphenol A, Lot No. 151-126-200, CAS No. 80-05-7, reported to have a radiochemical purity of 99.8%, were received from Moravek Biochemicals on 12 January and 16 February 2007.

[¹²C]Bisphenol A, Lot No. B0070138, CAS No. 80-05-7, used as an analytical standard, was received from Research Triangle Institute on 26 October 2004.

TEST END POINTS: Percent emergence and dry shoot weight

APPLICATION OF TEST SUBSTANCE: Mixed into sandy loam

TEST SPECIES: Cabbage (*Brassica oleracea*)
Corn (*Zea mays*)
Oat (*Avena sativa*)
Soybean (*Glycine max*)
Tomato (*Lycopersicon esculentum*)
Wheat (*Triticum aestivum*)

EFFECT CRITERIA: Percent emergence and dry shoot weight, and treatment-related morphological abnormalities were determined for each species.

NOMINAL TEST CONCENTRATIONS: 150 and 1000 mg/kg

**MEASURED
CONCENTRATIONS:**

Each test chemical stock solution used for preparation of the soil treatments was analyzed both radiometrically and by HPLC/UV methods. Portions of the treated soil samples were also analyzed radiometrically. Initial bisphenol A concentrations in the treated soils were calculated based on the specific activity of the test chemical stock solution. The measured concentrations indicated the stock solutions and soil concentrations closely approximated the desired nominal concentrations. Therefore, nominal concentrations were used to express the results of this study.

**DATES OF DEFINITIVE TESTS
(including dry weights):**

19 February to 21 March 2007

RESULTS:

Species	Nominal Concentration (mg/kg)	Percent Inhibition ^a	
		Emergence	Dry Shoot Weight
Cabbage	150	57	1
	1000	100	100
Corn	150	5	46
	1000	10	98
Oat	150	13	27
	1000	21	95
Soybean	150	-3	9
	1000	8	79
Tomato	150	64	68
	1000	94	100
Wheat	150	-17	50
	1000	23	98

^a Percent inhibition relative to the appropriate control.

1.0 INTRODUCTION

The objective of this study was to determine the effects of bisphenol A at 150 and 1000 mg/kg on seedling emergence and early growth of six economically important, agricultural plant species. The test concentrations selected for this exposure were selected by the Study Sponsor.

The study was initiated on 31 January 2007, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The tests were conducted from 19 February to 21 March 2007 at Springborn Smithers Laboratories (SSL) located in Wareham, Massachusetts. All raw data, the protocol and the original final report produced during this study are stored in Springborn Smithers' archives at the above location.

2.0 MATERIALS AND METHODS

2.1 Protocol

The procedures followed during this study are described in the Springborn Smithers Laboratories protocol entitled "Seedling Emergence and Seedling Growth Test Following OECD Draft Guideline #208", Springborn Smithers Laboratories Protocol No.: 101006/OECD/Emergence and Growth/6 species/BPA (Appendix 1).

2.2 Test Substances

A series of stock solutions, containing mixtures of radiolabeled (^{14}C) and nonradiolabeled (^{12}C) bisphenol A dissolved in acetone, were received on 12 January and 16 February 2007 from Moravek Biochemicals, Brea, California. The following information was provided:

Name:	bisphenol A, [ring- ^{14}C]
Synonym:	[^{14}C]BPA
Lot No.:	151-126-200
CAS No.:	80-05-7
Radiochemical Purity:	99.8% (provided by Supplier)
Amount Received:	60 to 120 μCi aliquots

Procedures used in the preparation of the test chemical stock solutions and the results of the analysis performed by Moravek Biochemicals are provided in Appendix 3.

Upon receipt at Springborn Smithers, the test substance (SSL No. 121-23 to 121-27, 121-29 to 121-34, and 122-16) was stored in a freezer (-70 to -90 °C) in the original container and was used to prepare the test soils.

Nonradiolabeled bisphenol A used as a standard for the HPLC analysis of the test chemical stock solutions by Springborn Smithers, was received on 26 October 2004 from Research Triangle Institute, Research Triangle Park, North Carolina. The following information was provided:

Name:	Bisphenol A
Synonym:	BPA
Lot No.:	B0070138
CAS No.:	80-05-7
Purity:	99.62% (Appendix 2)
Date of Analysis:	11 October 2006 (most recent purity analysis for master Lot No. B0070138)
Expiration Date:	Stable, no expiration date assigned (per Study Sponsor)

Upon receipt at Springborn Smithers, the test substance (SSL No. 108-53) was stored at room temperature in the original container in a dark ventilated cabinet. This sample was used to prepare analytical standards. Concentrations were adjusted for the purity of the test substance and are presented as active ingredient (a.i.).

Determination of stability and characterization, verification of the test substance identity, maintenance of records on the test substance, and archival of a sample of the test substance are the responsibility of the Study Sponsor.

2.3 Test Species

The plant species tested were three monocotyledons, oats (*Avena sativa*), wheat (*Triticum aestivum*), and corn (*Zea mays*), and three dicotyledons, cabbage (*Brassica oleracea*), soybean (*Glycine max*) and tomato (*Lycopersicon esculentum*). The seeds had not been pretreated with

fungicides or insecticides. Seed variety, source, lot number, dates on which the seeds were packed and received, and the germination percentages for the seeds used during the seedling emergence tests are presented in Table 1. Upon receipt at Springborn Smithers, seeds were stored refrigerated at approximately 2 to 8 °C in the dark until test initiation.

2.4 Support Medium - Analyses and Characterization

Sandy loam collected from Fairhaven, Massachusetts (SSL Lot No. 063001) was purchased from Medeiros and Sons Trucking Company, Fairhaven, Massachusetts on 30 June 2001. The sandy loam was characterized by Agvise Labs, Northwood, North Dakota as containing 85% sand, 12% silt, 3% clay, with an organic carbon content of 1.1% (1.9% organic matter). A representative sample of the support medium was analyzed for the presence of pesticides, PCBs, and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were detected at concentrations that would compromise the results of this study (ASTM, 2002). The soil was heat-sterilized prior to use.

2.5 Exposure System

The exposure vessels consisted of polypropylene pots (Kord Products Ltd.). For cabbage, corn, soybean, oats and tomato, ten replicate pots were maintained for the control and each concentration tested. For wheat, five replicate pots were maintained for the control and each concentration tested. Each pot was 13-cm tall with a top diameter of 13 cm and a bottom diameter of 9 cm. The interior base was fitted with 20-cm diameter filter paper to retain the support medium and allow for plant uptake of the nutrient solution by subirrigation. The filter paper was added and then each pot was filled to a depth of 10 cm with 1.2 kg of support medium. Each pot was placed in a polypropylene saucer (Kord Products Ltd.) and received approximately 100 mL of nutrient solution via sub-irrigation.

The study was conducted in a greenhouse designed as follows: whenever natural light intensity fell below approximately 800 footcandles (8600 lux), sodium vapor lights supplemented natural light when necessary to maintain > 800 footcandles during the light period (photoperiod 16 hours

light/8 hours dark). The temperature was maintained between 15 to 36 °C, heating and cooling cycled as required.

The potential for air pollution within the greenhouse is believed to be minimal due to the rural location of the laboratory and the lack of other industrial businesses in the area. The greenhouse is located in a relatively isolated section of the laboratory grounds, which reduces the possibility of air contamination from concurrent testing.

2.6 Well Water and Nutrient Solution

Well water was used to water the plants. Routine analyses for the presence of pesticides, PCBs and toxic metals were conducted periodically by GeoLabs, Inc., Braintree, Massachusetts, on representative samples of the well water provided. None of these compounds were detected at concentrations that would compromise the results of the study (ASTM, 2002). Additionally, the well water was analyzed for the presence of residual bisphenol A by ABC Laboratories, Columbia, Missouri. The results indicated that the concentration of bisphenol A was below the limit of detection (e.g., 0.074 µg/L).

Additionally, the plants were subirrigated twice weekly with nutrient solution prepared from Peters 20-20-20 (SSL No. 22102, supplied by Griffin Greenhouse Supplier) dilute to 200 mg/L with well water. Approximately 100 mL was provided to all pots by sub-irrigation. All additional waterings were provided using well water.

2.7 Stock and Test Solution Preparation

The test chemical stock solutions were prepared by Moravek Biochemicals, Brea, California prior to shipping (Appendix 3). Different dosage solutions were prepared for each soil treatment by combining appropriate amounts of a 1 mCi/mL [¹⁴C]bisphenol A stock solution in acetone and varying amounts of [¹²C]bisphenol A. The materials were diluted with acetone to a total volume of 50 mL. The desired volumes and concentrations for each dosage solution were

provided to Moravsek Biochemicals by the Study Sponsor in consultation with the Study Director, and are presented in the following table:

Nominal BPA Conc. In Dry Soil (mg/kg)	Total mass of soil (kg) per batch to prepare	Fixed Amount of [¹⁴ C]BPA (mCi) per batch (6 or 12 kg) soil	Amount of [¹⁴ C]BPA per batch (6 or 12 kg) soil (mg)	Amount of [¹² C]BPA per batch of soil (mg)	Approximate Radioactivity in Soil (dpm/g)	Stock/Dosing Solution Volume (mL) to be Applied	Stock/Dosing Solution Conc. (mg/mL)	Number of Stocks to Prepare
1000	12	0.12	0.137	11999.86	22,000	50	240	5
1000	6	0.06	0.0684	5999.93	22,000	50	120	1
150	12	0.12	0.137	1799.86	22,000	50	36	5
150	6	0.06	0.0684	899.93	22,000	50	18	1

For the tests with cabbage, corn, soybean, oats and tomato, 50 mL of the appropriate stock solution was applied to 0.50 kg silica sand and the treated sand was placed in a fume hood to allow the acetone to evaporate. Once dry, the treated sand was dispersed into 11.5 or 12 kg (dry weight) of sandy-loam soil and mixed with a Hobart Mixer for 10 minutes to provide the desired nominal concentrations. For the test with wheat, a total of 6 kg of sandy loam soil was prepared using 0.50 kg silica sand and 5.5 kg (dry weight) of sandy loam soil as described above. The final nominal radioactivity of [¹⁴C]bisphenol A in the treated soil was approximately 22,000 dpm/g soil dry weight. Solvent control soil was prepared prior to and in the same manner as the treated soils (i.e., 50 mL acetone, applied to sand, evaporated and mixed in soil), but did not receive any test substance.

2.8 Test Initiation

The following table presents species replication and the number of seeds exposed per replicate during the study:

Species	Number of Replicates/Treatment	Number of Seeds/Replicate	Number of Seeds/Treatment
Cabbage	10	4	40
Corn	10	2	20
Oats	10	8	80
Soybean	10	2	20
Tomato	10	2	20
Wheat	5	8	40

The number of seeds selected per replicate was based on seed and seedling size. Approximately 1.2 kg of treated soil was added to each pot.

All pots were labeled to identify the plant species, nominal concentration, replicate and study number. Control pots contained untreated sterile, sandy-loam. The soil in each pot was leveled and the appropriate number of seeds were impartially selected and planted at a depth of approximately 1 to 2 cm in each pot (20, 40 or 80 seeds per treatment and controls, depending upon species). The seeds were placed in a circular pattern around the inside perimeter of the pot. To locate specific plants within the pot, the plant located nearest the pot label constituted plant number one. The remaining plant positions were determined sequentially in a clockwise order. Approximately 100 mL of nutrient solution was added to each saucer. Thereafter, nutrient solution was added twice weekly. Control replicates were planted first, then solvent control, followed by the treatment levels (low to high concentration). Pots were grouped by species and placed in a random block format based on computer-generated random numbers.

2.9 Test Monitoring

Air temperature was controlled using a thermostatically-regulated heating/cooling system and was constantly monitored using a Fisher Scientific minimum/maximum thermometer. Light intensity was measured daily using a Traceable radiometer/photometer held at average maximum leaf height for each species. Light intensity was measured in footcandles and converted to lux, based on 1 footcandle = approximately 10.76 lux. Humidity was maintained through evaporation of water from the irrigation solution, and was monitored using a Traceable Thermo-hygrometer.

Each control pot was observed daily until $\geq 50\%$ emergence was observed in the control. Seven, 14 and 21 days after 50% emergence in the control, the number of emerged plants, morphological abnormalities (e.g., chlorosis or necrosis of leaves) or mortalities were recorded. When $\geq 50\%$ emergence was not observed among the control plants, the solvent control plants were used to determine observation intervals. All control and treatment levels were terminated

21 days after $\geq 50\%$ emergence in the controls was determined. At test termination, the above ground portion of the live plants within a pot were removed, placed in pre-washed aluminum pans and dried in radiant heat ovens at $70 \pm 5^\circ\text{C}$ for at least three days before determining dry shoot weights to the nearest 0.0001 g.

2.10 Analytical Measurements

Prior to test initiation, a sample of each test substance dosing solution was taken for confirmation of total [^{14}C]radioactive concentration. Samples of each dosing solution were mixed with liquid scintillation cocktail (UltimaGold, Perkin Elmer Life Sciences) and measured by Liquid Scintillation Counting (LSC). Additionally, a second sample of each stock solution was collected, diluted into the calibration standard range with acetonitrile, and analyzed by high performance liquid chromatography with ultraviolet detection (HPLC/UV) procedures to determine the bisphenol A concentration according to the following instrumental conditions:

Instrument:	Hewlett Packard quaternary solvent pump Series 1100 equipped with a Hewlett Packard Series 1100 degasser and autosampler, a Hewlett Packard diode array detector, and Hewlett Packard ChemStation Version A.06.03 for data acquisition		
Column:	Agilent SB-C18, 3.5 μm , 75 mm x 4.6 mm,		
Mobile Phase (A):	0.05% phosphoric acid in reagent water		
Mobile Phase (B):	100% acetonitrile		
Gradient:	<u>Time (min.)</u>	<u>Solvent A</u>	<u>Solvent B</u>
	0.00	95.0	5.0
	2.00	95.0	5.0
	12.0	0.00	100.0
	14.0	0.00	100.0
	15.0	95.00	5.0
Flow Rate:	1.4 mL/minute		
Injection Volume:	10 μL		
Wavelength:	230 nm		
Column Temperature:	ambient		
Run Time:	15 minutes		
Equilibration Delay:	3.00 minutes		
Retention Time:	approximately 8.2 minutes		

Measured concentrations of bisphenol A were determined using a linear regression calibration curve. Calibration standards were prepared in acetonitrile at concentrations of 10.0, 15.0, 25.0, 50.0, 75.0 and 100 mg/L.

Initial test substance concentrations were determined by radiometric analysis of the treated soils. On day 1, a sample of treated soil was removed from two pots of each treatment level and the controls for each species. A known weight of each sample was then placed in cellulose combustion cones (CombustoCone, Perkin Elmer Life Sciences), air dried, and combusted in a Harvey Model OX500 sample oxidizer to release the total [^{14}C]bisphenol A. The captured $^{14}\text{CO}_2$ was mixed with LSC cocktail and measured by LSC. The concentration of total bisphenol A was calculated based on the measured specific activity of each stock solution added to the soil. Percent moisture was determined on a separate aliquot of each soil sample. Measured soil concentrations were adjusted for percent moisture and expressed as mg bisphenol A/kg dry soil.

The test substance concentrations in the soil treatments were intended to be analyzed at test initiation. Duplicate samples were removed for this purpose from each batch of treated soil. However, because the individual samples were subsequently and inadvertently composited by treatment, they were not analyzed.

2.11 Statistical Analysis

A t-Test (Sokal and Rohlf, 1981) was conducted to statistically compare the final percent emergence or dry shoot weight of the control to the solvent control data. If no significant difference was determined, control and solvent control data were pooled for comparison to treatment data. If a difference was determined between the control and solvent control data, the solvent control was used for comparison with the treatment data. Percent inhibition of the treatment data was calculated relative to the appropriate control data. Negative percent inhibition reflects increased emergence or growth.

3.0 RESULTS AND DISCUSSION

3.1 Test Monitoring

A summary of the environmental conditions monitored during the definitive tests is presented in Table 2. The relative humidity ranged from 15 to 64% and temperature ranged from 15 to 36 °C. The OECD Guideline #208 recommends relative humidity and temperature ranges of 45 to 95% and 12 to 32 °C for greenhouse testing. Although the actual conditions exceeded the recommended ranges, the conditions maintained have been used in past studies and no negative impact on the plants has been observed.

3.2 Analytical Results

The analytical results are presented for the dosing stock solution analyses in Table 3. Dosing stock measurements conducted by Moravek resulted in concentrations that ranged from 98 to 117% of nominal concentration using a spectrophotometric method. The dosing stock measurements conducted by SSL ranged from 82.4 to 101% of nominal concentration using HPLC methodology, and from 76 to 124% of nominal concentrations using [¹⁴C] counts by LSC. The similarity of the three measurements of each stock solution sample, indicates that the stock solutions were prepared correctly and contained the appropriate amount of bisphenol A.

The results of the day 1 soil analyses for bisphenol A concentration based on radiometric counts are presented in Table 4. Recoveries of replicate samples (N= 24) ranged from 80 to 126% of nominal concentration with a few exceptions. Two recoveries were 67 and 73% of nominal concentration and two were 141 and 180% of nominal concentration. The average recovery per treatment and per species ranged from 78 to 152% of nominal concentration. Overall, these results indicate each batch of soil was dosed with the appropriate stock solution and the dispersion of the test substance was within expectations for mixing a solid substance (e.g., treated sand) into soil.

3.3 Biological Effects

The morphological abnormalities (e.g., chlorosis or necrosis of leaves) and mortality observed during the study, and the percent emergence and dry shoot weights determined at test termination for each species are presented in Table 5 through Table 22. A summary of the percent inhibition for percent emergence and dry shoot weight for each species is presented in Table 23. The effects of bisphenol A on each species are discussed in the following sections. To minimize the redundancy, where pooled control data is mentioned, the control and solvent control data were determined to be statistically similar based on a t-Test ($p \leq 0.05$). If the control and solvent control data were statistically different, the treatment data were compared to the solvent control data.

Additionally, please note that negative inhibition throughout the text represents increased growth relative to the appropriate control data.

Cabbage - The morphological abnormalities and mortality observed at test termination are presented in Table 5. No dead plants or plants with morphological abnormalities were observed among plants in the control, solvent control, or the 150 mg/kg treatment. Seeds exposed to the 1000 mg/kg treatment did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 6 and Table 7, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 48 and 70%, respectively. The average control and solvent control percent emergence, 59%, is slightly less than the minimum of 70% requested in the OECD Guideline #208. The seeds were packed in 2004 for the 2005 growing season and used in February 2007. The percent emergence (59%) observed in this test was equivalent to that observed in the subsequent definitive test with cabbage (SSL Study No. 13761.6124) using new cabbage seed packed for the 2007 growing season, indicating the age of the seed was not the reason for low percent emergence. Although the percent emergence was less than requested by the study guideline, the response to the test substance was adequate to establish definitive test concentrations at an appropriate range to

define NOEC and EC50 values. The day 21 mean percent seedling emergence for the 150 and 1000 mg/kg treatments was 30 and 0%, respectively, and yielded 57 and 100% inhibition, respectively, relative to the solvent control data. The day 21 mean shoot dry weight for the control and solvent control were 0.2984 and 0.2740 g, respectively (pooled control = 0.2855 g). The day 21 shoot dry weight for the 150 and 1000 mg/kg treatments was 0.2818 g and 0 g, respectively, and yielded 1 and 100% inhibition, respectively, relative to the pooled control data.

Corn - The morphological abnormalities and mortality observed at test termination are presented in Table 8. No dead plants or plants with morphological abnormalities were observed in the controls or the 150 mg/kg treatment. Most plants exposed to the 1000 mg/kg treatment level were observed to be necrotic at test termination. One and two seeds exposed to the 150 and 1000 mg/kg treatment levels, respectively, did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 9 and Table 10, respectively. The day 21 mean percent seedling emergence was 100% for both the control and solvent control (pooled control = 100%). The day 21 mean percent seedling emergence for the 150 and 1000 mg/kg treatments was 95% and 90%, respectively, and yielded 5 and 10% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control was 1.3601 and 1.3094 g, respectively (pooled control = 1.3348 g). The day 21 shoot dry weight for the 150 and 1000 mg/kg treatments was 0.7242 g and 0.0286 g, respectively, and yielded 46 and 98% inhibition, respectively, relative to the pooled control data.

Oat - The morphological abnormalities and mortality observed at test termination are presented in Table 11. One dead plant was observed in each of the control and 1000 mg/kg treatment level, but no morphological abnormalities were noted. No dead plants or plants with morphological abnormalities were observed in the solvent control or the 150 mg/kg treatment level. Several seeds exposed to the controls and the treatment levels did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 12 and Table 13, respectively. The

day 21 mean percent seedling emergence for the control and solvent control was 84 and 94%, respectively (pooled control = 89%). The day 21 mean percent seedling emergence for the 150 and 1000 mg/kg treatments was 78% and 70%, respectively, and yielded 13 and 21% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control were 0.2106 and 0.1962 g, respectively (pooled control = 0.2038 g). The day 21 shoot dry weight for the 150 and 1000 mg/kg treatments was 0.1487 and 0.0092 g, respectively, and yielded 27 and 95% inhibition, respectively, relative to the pooled control data.

Soybean - The morphological abnormalities and mortality observed at test termination are presented in Table 14. No dead plants or plants with morphological abnormalities were observed in the controls or the 150 mg/kg treatment level. Two plants exposed to the 1000 mg/kg treatment level were observed to be necrotic at test termination. One and two seeds exposed to the solvent control and the 1000 mg/kg treatment, respectively, did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 15 and Table 16, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 100 and 95%, respectively (pooled control = 98%). The day 21 mean percent seedling emergence for the 150 and 1000 mg/kg treatments was 100% and 90%, respectively, and yielded -3 (increased) and 8% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control were 1.1321 and 1.0970 g, respectively (pooled control = 1.1145 g). The day 21 shoot dry weight for the 150 and 1000 mg/kg treatments was 1.0132 g and 0.2293 g, respectively, and yielded 9 and 79% inhibition, respectively, relative to the pooled control data.

Tomato - The morphological abnormalities and mortality observed at test termination are presented in Table 17. No dead plants were observed in the controls or the treatment levels tested. However, several seeds exposed to the controls and the 150 mg/kg treatment did not emerge by test termination. One plant exposed to the 1000 mg/kg treatment was observed to be necrotic at test termination, no other seeds emerged in the 1000 mg/kg treatment.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 18 and Table 19, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 85 and 80%, respectively (pooled control = 83%). The day 21 mean percent seedling emergence for the 150 and 1000 mg/kg treatments was 30% and 5%, respectively, and yielded 64 and 94% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control were 0.7933 and 0.5226 g, respectively. The day 21 shoot dry weight for the 150 and 1000 mg/kg treatments was 0.1681 and 0.0005 g, respectively, and yielded 68 and 100% inhibition, respectively, relative to the solvent control data.

Wheat - The morphological abnormalities and mortality observed at test termination are presented in Table 20. One and two dead plants were observed in the control and the 1000 mg/kg treatment level, respectively. No dead plants or plants with morphological abnormalities were observed in the solvent control or the 150 mg/kg treatment level. Two seeds exposed to the 150 mg/kg treatment level did not emerge by test termination. Several seeds exposed to the control, solvent control and 1000 mg/kg treatment level did not emerge.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 21 and Table 22, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 93 and 73%, respectively (pooled control = 83%). The day 21 mean percent seedling emergence for the 150 and 1000 mg/kg treatments was 93% and 63%, respectively, and yielded -17 (increase) and 23% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control were 0.1671 and 0.1463 g, respectively (pooled control = 0.1567 g). The day 21 shoot dry weights for the 150 and 1000 mg/kg treatments was 0.0784 g and 0.0035 g, respectively, and yielded 50 and 98% inhibition, respectively, relative to the pooled control data.

4.0 CONCLUSIONS

Bisphenol A elicited effects to the six plant species tested, cabbage, corn, oat, soybean, tomato and wheat. Dry shoot weight was a more sensitive indicator of the effects of bisphenol A than percent emergence, with the exception of cabbage and tomato which demonstrated equal sensitivity at both parameters in the 1000 mg/kg treatment. The percent inhibition of percent emergence and dry shoot weight determined in the 150 and 1000 mg/kg treatments in summarized in Table 23. A second series of exposures were conducted with these six species to determine EC25, EC50, NOEC and LOEC values for bisphenol A under Springborn Smithers Study No. 13761.6124.

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**SPRINGBORN SMITHERS
LABORATORIES**

PROTOCOL DEVIATION

The protocol states that two samples of treated soil will be collected from each batch (e.g., 6 or 12 kg) for each concentration and the controls directly after the batch of soil has been mixed thoroughly to obtain homogeneous distribution of the test item. Samples were collected in this manner, but later each batch was inadvertently combined by treatment. Therefore, additional samples were collected directly from the pots on days 1 and 3. On day 1, a sample was collected from two individual pots for each treatment or control and for each species tests. On day 3, additional samples were collected from each treatment and control, and species, as archive samples and held frozen for analyses if deemed necessary. The analytical results for the soil analyses presented in this report are based on the analysis of the day 1 soil samples. Since the results of these analyses in general closely approximated the expected nominal concentrations, this deviation did not impact the results of the study.

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Table 1. Historical data for seeds used in the definitive seedling emergence and growth tests with bisphenol A.

Species ^a	Variety ^a	Date Packed ^a	Date Received	Supplier Lot No. ^a	SSL Lot No.	% Germ.	Date Tested
Cabbage	Ruby Perfection ^b	6/04	9/29/04	HU12	92904D	98	8/04
Corn	Truckers Favorite ^c	NA ^f	10/5/06	NA	100506	90	11/05
Oat	Jerry ^d	2006	10/10/06	05TI	101006C	98	12/05
Soybean	Edible Early Hakucho ^b	NA	10/10/06	AU04	101006A	96	4/06
Tomato	Celebrity Hybrid ^b	NA	6/8/06	NA	060806	NA	NA
Wheat	VNS ^e	NA	12/18/06	TRAR-35927	121806	96	8/1/06

^a Information provided by the supplier.^b Supplied by Park Seed Company, Greenwood, South Carolina.^c Supplied by Carolina Biological Supply Company, Burlington, North Carolina.^d Supplied by Seeds of Change, Santa Fe, New Mexico.^e Supplied by Granite Seed Company, Lehi, Utah.^f NA = Not Applicable.

All seeds refrigerated at approximately 4 °C in the dark until test initiation.

Table 2. Environmental conditions measured in the greenhouse during the seedling emergence and growth tests with bisphenol A.

Parameter	Range ^a
Relative Humidity ^b (%)	15 - 64
Temperature ^b (°C)	15 - 36
Light Intensity ^c (ftc)	600 - 2800
Light Intensity ^c (lux)	6500 - 30,000

^a Rounded to two significant figures.

^b The OECD Guideline #208 recommends relative humidity and temperature ranges of 45 to 95% and 12 to 32 °C for greenhouse testing. Although the actual conditions exceeded the recommended ranges, the conditions maintained have been used in past studies and no negative impact on the plants has been observed.

^c For light intensity, overall range was based on daily readings taken in multiple locations in the greenhouse.

Table 3. Summary of the stock solution analysis for the dosing stocks used during the seedling emergence and growth tests with bisphenol A.

Moravek Stock Solution ID	Nominal Stock Conc. (mg/mL)	Moravek Measured Stock Conc. ^a (mg/mL)	Percent Nominal (%)	SSL TMC No.:	SSL Measured Stock Conc. (mg/mL) (HPLC)	Percent Nominal (%) (HPLC)	SSL Measured Stock Conc. ^b (mg/mL) [¹⁴ C]	Specific Activity (dpm/μg)
NA ^c	NA	NA	NA	NA	NA		NA	
34R	18	20.1	112	122-16	18.0	100	13.6	112.11
29	36	40.0	111	121-24	36.3	101	37.9	154.63
32	36	37.8	105	121-25	36.0	99.9	44.8	184.28
31	36	42.2	117	121-26	35.9	99.7	44.1	181.61
30	36	39.4	109	121-29	36.3	101	33.0	134.63
33	36	39.8	111	121-33	35.8	99.4	42.3	174.84
28	120	136	114	121-23	113	94.1	103	20.284
26	240	246	102	121-27	200	83.4	225	24.942
27	240	246	102	121-30	203	84.5	234	25.667
25	240	246	103	121-31	21	83.8	191	21.039
24	240	236	98	121-32	236	98.3	195	18.324
23	240	240	100	121-34	198	82.4	229	25.700

^a The stock concentrations were verified by spectrophotometric analysis at Moravek Biochemicals before shipment to Springborn Smithers Laboratories (SSL). Stock solution vials were not clearly labeled by replicate, so the measurements from Moravek for the 36 and 240 mg/mL solutions may not directly correlate with the replicate measurements made at SSL.

^b Concentrations of bisphenol A determined by [¹⁴C] were calculated based on the unique specific activity calculated for each stock solution.

^c NA = Not Applicable.

Table 4. Summary of test day 1 soil analyses based on radiometric counts during the seedling emergence and growth tests with bisphenol A.

Species- Soil Treated	Nominal Concentration (mg/kg)	Day 1 Measured Conc. ^a (mg/kg) [¹⁴ C]	Average Measured Concentration (mg/kg)	Percent Nominal (%) [¹⁴ C]	Average Percent Nominal (%) [¹⁴ C]
S Ctrl A	NA ^b	<0.99		NA	
S Ctrl B	NA	<0.98	NA	NA	NA
cabbage A	150	133		88	
cabbage B	150	137	135	91	90
corn A	150	151		101	
corn B	150	157	154	105	103
oat A	150	137		92	
oat B	150	109	123	73	82
soybean A	150	133		89	
soybean B	150	100	117	67	78
tomato A	150	153		102	
tomato B	150	121	137	80	91
wheat A	150	140		93	
wheat B	150	135	137	90	92
cabbage A	1000	890		89	
cabbage B	1000	1163	1030	116	103
corn A	1000	917		92	
corn B	1000	797	857	80	86
oat A	1000	1799		180	
oat B	1000	1236	1520	124	152
soybean A	1000	1265		126	
soybean B	1000	1414	1340	141	134
tomato A	1000	1112		111	
tomato B	1000	955	1030	96	103
wheat A	1000	976		98	
wheat B	1000	990	983	99	98

^a Concentrations of bisphenol A determined by ¹⁴C were calculated from radioactivity measured in the soil samples and the unique measured specific activity for each stock solution, based on the following formula. Samples were collected directly from pots as indicated by species and replicate.

$$A = \frac{\text{dpm}}{\text{SW}} \times \frac{1}{\text{SA}}$$

where:

- dpm = disintegrations per minute, radioactivity corresponding to the HPLC peak area of [¹⁴C]bisphenol A (determined by the division of the cpm generated by the detector by the dpm factor), or the total [¹⁴C] residue content of the extracts
- SW = injection volume (mL) for HPLC/RAM, or sample weight (kg) for total [¹⁴C] residue analysis of the extracts by LSC
- SA = effective specific activity for bisphenol A (specific activity x percent radiolabeled as a decimal)
- A = analytical result (mg/kg), concentration in the original sample

^b NA = Not Applicable.

NOTE: Soil concentrations are based on mg/kg dry weight of soil.

Table 5. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for cabbage (*Brassica oleracea*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Plant Condition at Test Termination										
	Plant Number	1	2	3	4	Replicate		7	8	9	10
Control	1	H	H	H	H	H	H	H	H	H	Ne
	2	Ne	H	H	H	Ne	H	H	H	Ne	Ne
	3	Ne	H	Ne	H	Ne	Ne	H	H	Ne	Ne
	4	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
Solvent Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	Ne	H	Ne	H	Ne	H
	4	Ne	Ne	H	Ne	Ne	Ne	Ne	Ne	Ne	Ne
150	1	H	H	H	Ne	H	H	H	H	H	H
	2	Ne	Ne	Ne	Ne	H	H	Ne	H	Ne	Ne
	3	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	4	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
1000	1	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	2	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	3	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	4	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne

H = Healthy
 Ne = Non-emerged
 N = Necrotic
 Dp = Dead plant

Table 6. Percent emergence of cabbage (*Brassica oleracea*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	3	25	48	NA ^b
	2	1	75		
	3	2	50		
	4	1	75		
	5	3	25		
	6	2	50		
	7	1	75		
	8	1	75		
	9	3	25		
	10	4	0		
Solvent Control	1	1	75	70	NA
	2	1	75		
	3	0	100		
	4	1	75		
	5	2	50		
	6	1	75		
	7	2	50		
	8	1	75		
	9	2	50		
	10	1	75		
150	1	3	25	30	57
	2	3	25		
	3	3	25		
	4	4	0		
	5	2	50		
	6	2	50		
	7	3	25		
	8	2	50		
	9	3	25		
	10	3	25		
1000	1	4	0	0	100
	2	4	0		
	3	4	0		
	4	4	0		
	5	4	0		
	6	4	0		
	7	4	0		
	8	4	0		
	9	4	0		
	10	4	0		

^a Percent inhibition relative to the solvent control.

^b NA = Not Applicable.

Table 7. Shoot dry weight of cabbage (*Brassica oleracea*) exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	0.4618	NA ^d	0.2984	0.1059	NA
	2	0.2812	0.1873			
	3	0.3910	0.0107			
	4	0.2093	0.0583			
	5	0.3885	NA			
	6	0.2401	0.2863			
	7	0.2135	0.1191			
	8	0.1431	0.2005			
	9	0.3568	NA			
	10	NA	NA			
Solvent Control	1	0.2103	0.0987	0.2740	0.0941	NA
	2	0.2220	0.1676			
	3	0.1888	0.0252			
	4	0.2077	0.0867			
	5	0.2449	0.3402			
	6	0.2450	0.0463			
	7	0.3746	0.0446			
	8	0.2949	0.1992			
	9	0.4441	0.1630			
	10	0.3075	0.1227			
Pooled Control 150	1	0.2889	NA	0.2855 0.2818	0.0923 0.1425	NA 1
	2	0.5738	NA			
	3	0.3017	NA			
	4	NA	NA			
	5	0.2232	0.2700			
	6	0.2306	0.0479			
	7	0.2151	NA			
	8	0.2616	0.0276			
	9	0.3916	NA			
	10	0.0501	NA			
1000	1	NA	NA	0.0000	NA	100
	2	NA	NA			
	3	NA	NA			
	4	NA	NA			
	5	NA	NA			
	6	NA	NA			
	7	NA	NA			
	8	NA	NA			
	9	NA	NA			
	10	NA	NA			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

Table 8. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for corn (*Zea mays*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Plant Condition at Test Termination												
	Plant Number	1	2	3	4	Replicate		5	6	7	8	9	10
Control	1	H	H	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H	H	H
Solvent Control	1	H	H	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H	H	H
150	1	H	H	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H	Ne	H
1000	1	N	N	N	N	N	N	N	N	N	N	N	H
	2	N	Ne	N	N	N	N	N	N	N	N	Ne	H

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 9. Percent emergence of corn (*Zea mays*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	0	100	100	NA ^b
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
Solvent Control	1	0	100	100	NA
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
Pooled Control	NA	NA	NA	100	NA
150	1	0	100	95	5
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	1	50		
	10	0	100		
1000	1	0	100	90	10
	2	1	50		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	1	50		
	10	0	100		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.

Table 10. Shoot dry weight of corn (*Zea mays*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Shoot Dry Weight (g)					
	Replicate	Day 21 Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	1.7448	0.3011	1.3601	0.3900	NA ^d
	2	1.8588	0.8394			
	3	1.1836	1.2881			
	4	1.2119	1.3635			
	5	1.4094	0.2142			
	6	2.0307	0.1643			
	7	1.1241	0.1856			
	8	1.1444	0.1363			
	9	1.0551	0.1438			
	10	0.8390	0.4219			
Solvent Control	1	1.3285	0.2596	1.3094	0.2536	NA
	2	1.5036	0.1187			
	3	1.5536	0.0191			
	4	1.4135	0.2752			
	5	0.7818	0.6194			
	6	1.1235	0.3929			
	7	1.1618	0.4442			
	8	1.3717	1.3215			
	9	1.6555	0.4097			
	10	1.2006	0.9865			
Pooled Control 150	1	0.2992	0.0116	1.3348 0.7242	0.3212 0.2473	NA 46
	2	0.9186	0.3688			
	3	0.7843	0.2617			
	4	0.7557	0.0860			
	5	0.8519	0.1341			
	6	0.5265	0.2172			
	7	0.8275	0.2603			
	8	0.7836	0.0793			
	9	1.1057	NA			
	10	0.3889	0.3987			
1000	1	0.0343	0.0001	0.0286	0.0153	98
	2	0.0532	NA			
	3	0.0374	0.0179			
	4	0.0082	0.0067			
	5	0.0182	0.0181			
	6	0.0107	0.0122			
	7	0.0234	0.0193			
	8	0.0168	0.0103			
	9	0.0461	NA			
	10	0.0374	0.0180			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

Table 11. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Plant Condition at Test Termination										
	Plant Number	1	2	3	4	Replicate		7	8	9	10
Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	H	H	H	H	H	H
	4	H	H	H	H	H	Dp	H	H	H	H
	5	H	H	H	H	H	H	H	H	H	H
	6	H	H	H	H	H	H	H	H	H	H
	7	H	H	H	H	H	H	H	H	H	Ne
	8	H	H	H	H	Ne	Ne	H	Ne	H	Ne
Solvent Control	1	H	H	H	H	H	H	H	H	H	Ne ^a
	2	H	H	H	H	H	H	H	H	H	Ne
	3	H	H	H	H	H	H	H	H	H	Ne
	4	H	H	H	H	H	H	H	H	H	Ne
	5	H	H	H	H	H	H	H	H	H	Ne
	6	H	H	H	H	H	H	H	H	H	Ne
	7	H	Ne	H	H	H	H	Ne	H	H	Ne
	8	H	Ne	H	H	H	H	Ne	Ne	H	Ne
150	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	H	H	H	H	H	H
	4	Ne	H	H	H	H	H	H	H	H	H
	5	Ne	H	H	H	H	H	H	H	H	H
	6	Ne	H	H	H	H	H	Ne	H	H	Ne
	7	Ne	Ne	Ne	H	H	Ne	Ne	H	H	Ne
	8	Ne	Ne	Ne	H	H	Ne	Ne	Ne	H	Ne
1000	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	H	H	H	H	H	H
	4	H	H	H	H	H	H	H	H	H	H
	5	Ne	H	H	H	H	Ne	H	H	H	H
	6	Ne	H	H	H	H	Ne	H	Ne	Ne	Ne
	7	Ne	H	Dp	Ne	H	Ne	Ne	Ne	Ne	Ne
	8	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

^a Solvent control replicate 10 apparently was not planted with seeds.

Table 12. Percent emergence of oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	0	100	84	NA ^b
	2	0	100		
	3	0	100		
	4	0	100		
	5	1	88		
	6	1	88		
	7	0	100		
	8	1	88		
	9	0	100		
	10	2	75		
Solvent Control	1	0	100	94	NA
	2	2	75		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	2	75		
	8	1	88		
	9	0	100		
	10	8	0		
Pooled Control		NA	NA	89	NA
150	1	5	38	78	13
	2	2	75		
	3	2	75		
	4	0	100		
	5	0	100		
	6	2	75		
	7	3	63		
	8	1	88		
	9	0	100		
	10	3	63		
1000	1	4	50	70	21
	2	1	88		
	3	1	88		
	4	2	75		
	5	1	88		
	6	4	50		
	7	2	75		
	8	3	63		
	9	3	63		
	10	3	63		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.

Table 13. Shoot dry weight of oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Shoot Dry Weight (g)					
	Replicate	Day 21 Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	0.1921	0.0344	0.2106	0.0196	NA ^d
	2	0.2004	0.0544			
	3	0.2078	0.0327			
	4	0.2028	0.0714			
	5	0.2164	0.0337			
	6	0.2580	0.1208			
	7	0.1971	0.0360			
	8	0.2138	0.0321			
	9	0.1935	0.0369			
	10	0.2239	0.0930			
Solvent Control	1	0.2092	0.0511	0.1962	0.0133	NA
	2	0.1838	0.0891			
	3	0.1757	0.0383			
	4	0.1966	0.0350			
	5	0.1843	0.0426			
	6	0.2098	0.0583			
	7	0.2134	0.0333			
	8	0.1912	0.0990			
	9	0.2016	0.0543			
	10	NA	NA			
Pooled Control 150	1	0.1058	0.0814	0.2038 0.1487	0.0180 0.0202	NA 27
	2	0.1895	0.0982			
	3	0.1563	0.0492			
	4	0.1469	0.0281			
	5	0.1491	0.0446			
	6	0.1554	0.0783			
	7	0.1469	0.0574			
	8	0.1414	0.0425			
	9	0.1456	0.0790			
	10	0.1504	0.0726			
1000	1	0.0081	0.0013	0.0092	0.0022	95
	2	0.0091	0.0054			
	3	0.0069	0.0015			
	4	0.0117	0.0041			
	5	0.0095	0.0025			
	6	0.0135	0.0044			
	7	0.0107	0.0041			
	8	0.0063	0.0020			
	9	0.0089	0.0053			
	10	0.0076	0.0018			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

Table 14. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for soybean (*Glycine max*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Plant Number	Plant Condition at Test Termination									
		1	2	3	4	Replicate		7	8	9	10
Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
Solvent Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	Ne	H	H	H	H
150	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
1000	1	H	H	H	H	H	H	N	H	H	N
	2	H	H	Ne	H	H	H	H	H	Ne	H

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 15. Percent emergence of soybean (*Glycine max*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	0	100	100	NA ^b
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
Solvent Control	1	0	100	95	NA
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	1	50		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
Pooled Control				98	NA
150	1	0	100	100	-3
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
1000	1	0	100	90	8
	2	0	100		
	3	1	50		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	1	50		
	10	0	100		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.

Table 16. Shoot dry weight of soybean (*Glycine max*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Shoot Dry Weight (g)					
	Replicate	Day 21 Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	1.3126	0.0156	1.1321	0.1640	NA ^d
	2	0.9851	1.3207			
	3	1.1691	0.6106			
	4	1.3382	0.3840			
	5	1.0195	0.0899			
	6	1.2254	0.4709			
	7	1.2818	0.4209			
	8	1.1647	0.0187			
	9	0.8773	0.0554			
	10	0.9471	0.6536			
Solvent Control	1	1.0131	0.3051	1.0970	0.3313	NA
	2	0.8649	0.0386			
	3	1.0234	0.1032			
	4	1.0964	0.5923			
	5	0.9536	0.2286			
	6	2.0248	NA			
	7	1.0312	0.3828			
	8	0.9859	0.0164			
	9	0.9904	0.1145			
	10	0.9865	0.0071			
Pooled Control 150	1	1.1997	0.1182	1.1145 1.0132	0.2551 0.1155	NA 9
	2	0.9016	0.0979			
	3	1.0006	0.2014			
	4	0.8865	0.3299			
	5	1.1436	0.1961			
	6	1.1546	0.1312			
	7	1.0410	0.3984			
	8	0.9481	0.2539			
	9	0.9186	0.6662			
	10	0.9381	0.0214			
1000	1	0.1788	0.0064	0.2293	0.0575	79
	2	0.1250	0.1027			
	3	0.2654	NA			
	4	0.1906	0.0148			
	5	0.1918	0.0056			
	6	0.3151	0.1659			
	7	0.2590	0.0993			
	8	0.2436	0.0349			
	9	0.2891	NA			
	10	0.2353	0.0363			

^a SD = Standard Deviation.

^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.

^c Percent inhibition relative to the pooled control.

^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

Table 17. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Plant Number	Plant Condition at Test Termination									
		1	2	3	4	Replicate		7	8	9	10
Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	Ne	H	Ne	H	Ne
Solvent Control	1	H	H	H	H	Ne	H	H	H	H	H
	2	Ne	H	H	H	Ne	H	H	H	Ne	H
150	1	Ne	H	H	H	H	Ne	Ne	H	H	Ne
	2	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
1000	1	Ne	Ne	Ne	Ne	Ne	Ne	Ne	N	Ne	Ne
	2	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 18. Percent emergence of tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	0	100	85	NA ^b
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	1	50		
	7	0	100		
	8	1	50		
	9	0	100		
	10	1	50		
Solvent Control	1	1	50	80	NA
	2	0	100		
	3	0	100		
	4	0	100		
	5	2	0		
	6	0	100		
	7	0	100		
	8	0	100		
	9	1	50		
	10	0	100		
Pooled Control				83	NA
150	1	2	0	30	64
	2	1	50		
	3	1	50		
	4	1	50		
	5	1	50		
	6	2	0		
	7	2	0		
	8	1	50		
	9	1	50		
	10	2	0		
1000	1	2	0	5	94
	2	2	0		
	3	2	0		
	4	2	0		
	5	2	0		
	6	2	0		
	7	2	0		
	8	1	50		
	9	2	0		
	10	2	0		

^a Percent inhibition relative to the pooled control.

^b NA = Not Applicable.

Table 19. Shoot dry weight of tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Shoot Dry Weight (g)					
	Replicate	Day 21 Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	0.7243	0.1078	0.7933	0.2256	NA ^d
	2	0.6377	0.0671			
	3	0.5810	0.2880			
	4	0.6028	0.0603			
	5	0.7103	0.0842			
	6	1.2786	NA			
	7	0.7920	0.0791			
	8	1.0399	NA			
	9	0.6343	0.3597			
	10	0.9322	NA			
Solvent Control	1	0.7219	NA	0.5226	0.2075	NA
	2	0.4994	0.3204			
	3	0.5092	0.2186			
	4	0.4111	0.0170			
	5	NA	NA			
	6	0.6575	0.0578			
	7	0.1916	0.0957			
	8	0.4146	0.0819			
	9	0.8942	NA			
	10	0.4036	0.1630			
150	1	NA	NA	0.1681	0.0812	68
	2	0.2610	NA			
	3	0.1114	NA			
	4	0.2010	NA			
	5	0.2323	NA			
	6	NA	NA			
	7	NA	NA			
	8	0.0423	NA			
	9	0.1604	NA			
	10	NA	NA			
1000	1	NA	NA	0.0005	NA	100
	2	NA	NA			
	3	NA	NA			
	4	NA	NA			
	5	NA	NA			
	6	NA	NA			
	7	NA	NA			
	8	0.0005	NA			
	9	NA	NA			
	10	NA	NA			

^a SD = Standard Deviation.

^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.

^c Percent inhibition relative to the solvent control.

^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

Table 20. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for wheat (*Triticum aestivum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Plant Condition at Test Termination					
	Plant Number	1	2	Replicate 3	4	5
Control	1	H	H	H	H	Dp
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	Ne
	7	H	H	H	H	Ne
	8	H	H	H	H	Ne
Solvent Control	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	Ne	H	Ne	Ne	H
	7	Ne	H	Ne	Ne	Ne
	8	Ne	H	Ne	Ne	Ne
150	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	Ne	H	H	H
	8	H	Ne	H	H	H
1000	1	H	H	H	H	H
	2	H	H	H	H	Dp
	3	H	H	H	H	H
	4	Ne	H	H	H	H
	5	Ne	Ne	H	Dp	H
	6	Ne	Ne	H	Ne	H
	7	Ne	Ne	H	Ne	Ne
	8	Ne	Ne	Ne	Ne	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 21. Percent emergence of wheat (*Triticum aestivum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	0	100	93	NA ^b
	2	0	100		
	3	0	100		
	4	0	100		
	5	3	63		
Solvent Control	1	3	63	73	NA
	2	0	100		
	3	3	63		
	4	3	63		
	5	2	75		
Pooled Control				83	NA
150	1	0	100	93	-17
	2	2	75		
	3	0	100		
	4	0	100		
	5	0	100		
1000	1	5	38	63	23
	2	4	50		
	3	1	88		
	4	3	63		
	5	2	75		

^a Percent inhibition relative to the pooled control.

^b NA = Not Applicable.

Table 22. Shoot dry weight of wheat (*Triticum aestivum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg/kg)	Shoot Dry Weight (g)					Inhibition (%) ^c
	Replicate	Day 21 Replicate Mean	SD ^a	Treatment Mean ^b	SD	
Control	1	0.1559	0.0841	0.1671	0.0336	NA ^d
	2	0.1413	0.0117			
	3	0.1596	0.0226			
	4	0.1527	0.0681			
	5	0.2260	0.0828			
Solvent Control	1	0.1622	0.0816	0.1463	0.0131	NA
	2	0.1340	0.0320			
	3	0.1333	0.1105			
	4	0.1568	0.1254			
	5	0.1454	0.0787			
Pooled Control 150	1	0.0710	0.0483	0.1567 0.0784	0.0264 0.0171	NA 50
	2	0.0957	0.0419			
	3	0.0523	0.0265			
	4	0.0869	0.0385			
	5	0.0858	0.0223			
1000	1	0.0038	0.0019	0.0035	0.0007	98
	2	0.0034	0.0013			
	3	0.0029	0.0020			
	4	0.0031	0.0013			
	5	0.0045	0.0020			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

Table 23. Summary of percent inhibition results for percent emergence and dry shoot weight calculated during the seedling emergence and growth tests exposing six plant species to bisphenol A.

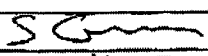

Species	Nominal Concentration (mg/kg)	Percent Inhibition ^a	
		Percent Emergence	Dry Shoot Weight
Cabbage	150	57	1
	1000	100	100
Corn	150	5	46
	1000	10	98
Oat	150	13	27
	1000	21	95
Soybean	150	-3	9
	1000	8	79
Tomato	150	64	68
	1000	94	100
Wheat	150	-17	50
	1000	23	98

^a Percent inhibition relative to the appropriate control.

APPENDIX 1 - STUDY PROTOCOL

TEST PROTOCOL

PROTOCOL TITLE: Seedling Emergence and Seedling Growth Test Following OECD
Draft Guideline #208

TO BE COMPLETED BY THE STUDY SPONSOR:	
Study Sponsor:	American Chemistry Council
Address:	1300 Wilson Blvd
	Arlington, VA 22209 Phone: 919-549-2236
Study Monitor:	Tilghman Hall E-mail: tilghman.hall@bayercropsscience.com
Study Sponsor Representative:	Steven Hentges E-mail: steve_hentges@plastics.org
Sponsor Protocol/Project No.:	
Test Substance Name(s): Bisphenol A	
Purity: 99.8%	Batch or Lot #: 151-126-200
Analytical Standard: NA	
Purity: NA	Batch or Lot #: NA
Additional Comments and Modifications:	
Sponsor Representative Approval:	 Date: 1/31/07
Study Monitor Approval:	 Date: 1/31/07

TO BE COMPLETED BY SPRINGBORN SMITHERS LABORATORIES BEFORE EXPERIMENT INITIATION:

Testing Facility: Springborn Smithers Laboratories 790 Main Street, Wareham, MA 02571-1037

Study Director: James Hoberg

Study No.: 13761.6123

Test Concentration: 150 and 1000 mg ai/Kg

Proposed Experimental Dates: (Start) 1 Feb 2007 (Termination) 25 Feb 2007


Study Director Signature31 Jan 2007
Study Initiation Date

* To be provided by protocol amendment, if applicable.

Springborn Smithers Protocol No.: 101006/OECD/Emergence and Growth/6 species/BPA Page 1 of 9

Seedling Emergence and Seedling Growth Test Following OECD Draft Guideline #208**1.0 OBJECTIVE**

The purpose of this study is to determine the effects of Bisphenol A at 150 and 1000 mg a.i./kg on the seedling emergence and early growth of six plant species. The number of emerged seedlings will be recorded 14 and 21 days after 50% of the control seedlings have emerged. Emergence is defined as the appearance of plant tissue above the surface of the support substrate. Observations will be recorded weekly for mortality and visual phytotoxicity (chlorosis, necrosis, etc.). At test termination, the number of emerged seedling, dry shoot weight and visual phytotoxicity will be recorded.

Replicate test and control pots will be within blocks in the greenhouse. The means and standard deviations will be calculated for control and treatment replicate measurements.

If a concentration response is observed, an EC50, EC25, and No-Observed-Effect Concentration (NOEC) if possible, will be determined for percent emergence and dry shoot weight. If less than 25% response is observed, the EC values will be stated as greater than the highest concentration tested.

2.0 MATERIALS AND METHODS**2.1 Chemical System****2.1.1 Test Substance**

The test substance will consist of radiolabeled and non-labeled Bisphenol A. Upon arrival at Springborn Smithers Laboratories, the test and reference substance(s) will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test substance will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

Each sample will be given a unique sample ID number and stored under the conditions specified by the Sponsor or manufacturer. The following information should be provided by the Study Sponsor, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

2.1.2 Test Substance Concentration Selection

The definitive test concentrations will be 150 and 1000 mg a.i./kg and were selected by the Study Sponsor. A negative control will be included and will consist of untreated soil.

A solvent control will also be included and will be prepared using an equal amount of organic solvent as applied to the treatments, but will not include test substance.

2.1.3 Solvent Control

A volatile organic solvent (e.g., acetone) will be used to solubilize the test substance. The solvent control will consist of organic solvent applied to silica sand, the solvent will be evaporated from the sand and the sand will be blended in the soil. Each treatment will be prepared in the same manner. An equal amount of solvent (50 mL/0.50 kg silica sand) will be used in the preparation of each treatment and the solvent control. The solvent control soil will be prepared followed by the treatments.

2.1.4 Stock Solutions and Exposure Soil Preparation

Different dosage solutions will be prepared for each soil treatment by combining a constant amount of ^{14}C -labelled test item and varying amounts of ^{12}C -labelled amounts of test item. The dosage solutions will be prepared by the supplier of the test item, and the specific activities (e.g. dpm per μmol) will be analysed and reported for each. For each treatment level, a dosage solution containing a mixture of ^{14}C -labelled and non-labelled test item will be dissolved in an accurately quantified volume of acetone sufficient to treat the corresponding batch of soil. The final nominal radioactivity of ^{14}C -labelled test item in the treated soil will be approximately 15000 dpm/g soil dry weight. The Study Director will provide the supplier of the test item with the desired volumes and concentrations for each dosage solution. The concentrations were selected by the Study Sponsor. The supplier of the test item will provide an appropriate volume of each dosage solution in a gas-tight container. The dosage solutions will be used immediately or stored in the freezer.

A Chemical Usage Log will also be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test substance is used. Stock solutions will be prepared by the supplier by dissolving the appropriate amounts of ^{14}C and ^{12}C test substance in a volatile organic solvent (acetone). The stock solution(s) will be shipped to Springborn Smithers and stored in properly labeled containers until needed. For the tests with cabbage, corn, soybean, oats and tomato, 50 mL of the appropriate stock solution will be applied to 0.50 kg silica sand and the sand will be placed in a fume hood until the solvent evaporates. The treated sand will then be dispersed into 11.5 kg (dry weight) of sandy loam soil and mixed with a Hobart mixer for ten minutes to provide the desired nominal concentrations. For the test with wheat, a total of 6 kg of sandy loam soil will be treated as noted above.

2.2 Test System

2.2.1 Species

The use of six test species helps to ensure that variations in seedling response to the test substance are detected. Recommended test species (US EPA, 1982 and OECD, 2003) are the following:

Monocotyledon:

Avena sativa – oats

Dicotyledon:

Brassica oleracea – cabbage

Monocotyledon:

Triticum aestivum – wheat
Zea mays – corn

Dicotyledon:

Glycine max – soybean
Lycopersicon esculentum – tomato

2.2.2 Justification of Test System

Selection of plant species for testing is based on several criteria including germination time, seedling size, sensitivity to chemical challenges and existing data base in toxicology studies.

2.2.3 Source

Seeds used for testing will not have been pretreated with fungicides or insecticides to avoid potential interactions with the test substance. The seed species, variety, source, lot number, and the germination percentage will be documented. The seeds will be purchased from a commercial supplier whose identity will be documented in the data and in the final report.

2.2.4 Irrigation

The plants will be irrigated using a commercially prepared water-soluble fertilizer containing essential major elements and micronutrients. The fertilizer used will be identified in the raw data and final report. The fertilizer will be provided to each replicate pot by subirrigation at a rate of approximately 100 mL twice weekly. All subsequent watering with well water will be on an as needed basis.

2.3 Physical System**2.3.1 Support Medium**

A local heat-sterilized sandy loam will be used as the support medium. The organic carbon content of the soil will be <1.5%. Soil moisture at the time of dosing will be approximately 10%. A representative sample of the sandy loam has been analyzed for the absence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, MA. Characterization of other physical parameters (i.e., particle size, cation exchange capacity) of the support medium has also been performed. Soil characterization will be provided in the final report. The support medium (approximately 1.2 kg, dry weight) will be contained within a polypropylene pot (top diameter = 13 cm, bottom diameter = 9 cm, height 13 cm, depth of medium = 10 cm).

2.3.2 Replication and Control of Bias

The following table presents species replication and number of seeds exposed.

Species	Number of Replicates	Number of Seeds/replicate
Cabbage	10	4
Wheat	5	8
Corn	10	2

Species	Number of Replicates	Number of Seeds/replicate
Soybean	10	2
Oats	10	8
Tomato	10	2

Treatment and control replicates will be positioned in a randomized block format based on computer-generated random numbers within the greenhouse.

2.3.3 Dilution Water

Periodic analyses of representative samples of the well water source used to prepare the nutrient solution are conducted by GeoLabs, Inc., Braintree, MA to ensure the absence of potential toxicants (e.g., pesticides, PCBs and selected toxic metals) at concentrations which may be harmful to the test organisms.

2.4 Test Procedures

2.4.1 Seedling Exposure Method

All pots will be labeled with the study number, test species, test concentration and replicate. Seeds will be planted approximately 1 to 2 cm below the surface of the support substrate. The exposure soil will be prepared as described in Section 2.1.4.

2.4.2 Environmental Conditions

Light intensity, relative humidity and temperature will be monitored and recorded daily throughout the test period. Whenever natural light falls below 800 foot-candles (8600 lux), sodium vapor lights will turn on until natural light is restored or until the end of the light period (16 hours light and 8 hours dark). The greenhouse temperature is generally expected to be 15 to 35 °C. Heating and cooling will cycle as required to maintain optimum growth.

2.4.3 Seedling Observations

Each control replicate will be observed four days after the exposure is initiated to determine the number of seedlings that have emerged. If $\geq 50\%$ emergence is not observed, the control replicates will be observed daily until this criterion is met. All plants will be observed weekly thereafter for visual phytotoxicity and mortality. Fourteen days after $\geq 50\%$ emergence is determined, the number of emerged plants will be recorded. The test will be terminated 21 days after $\geq 50\%$ emergence is determined in the control. At test termination, each replicate will be observed to determine the number of seedlings that have emerged. Any morphological abnormalities observed (e.g., chlorosis, necrosis) or mortalities will be recorded. At test termination, the above ground portion of the plants will be harvested. Plants will be placed in pre-tared containers (e.g., tins, bags) and will be dried at least three days at 70 ± 5 °C and weighed on an analytical balance.

Observation of morphological abnormalities will be evaluated with a rating scale based on the percentage of the plants exhibiting the abnormality. The rating scale will be from 0 to 100, where 0 indicates no injury or abnormalities and 100 indicates a dead plant.

2.5 Analytical Methodology

Three samples will be taken for confirmation of total ^{14}C -radioactive concentration of each dosage solution before use in the test.

Two samples of treated soil will be collected from each batch (e.g., 6 or 12 kg) for each concentration level and for the controls directly after the batch of treated soil has been mixed thoroughly to obtain homogeneous distribution of the test item. Soil samples of approximately 2 g (wet weight) will be collected and placed in a scintillation vial. A subsample will be transferred to a moisture balance and the percent moisture determined for that soil sample. If the samples for ^{14}C analysis are not analysed immediately, they will be stored frozen (approximately $-20\text{ }^{\circ}\text{C}$).

Total ^{14}C -radioactivity will be determined in each dosage solution before use in the test. The samples of the dosage solutions will be mixed with liquid scintillation cocktail (e.g. UltimaGold, PerkinElmer Life Sciences), and measured by Liquid Scintillation Counting (LSC; e.g. Beckman, model LS1801).

The soil samples will be thawed if necessary and each sample will be placed into a cellulose combustion cone (e.g. CombustoCone, PerkinElmer Life Sciences) and combusted in a sample oxidizer (e.g. Harvey, model OX500) to determine total ^{14}C -activity, mixed with LSC-cocktail (e.g. Permafluor, PerkinElmer), and measured by LSC.

Three quality control (QC) samples will be prepared, stored if necessary, and analyzed with the set of study samples. Results of these analyses indicate the accuracy of the analytical method for measuring test substance concentrations at each sampling period. The analytical method will be verified by Springborn Smithers Laboratories prior to test initiation.

3.0 DATA ANALYSIS

Test data will be presented in tabular format that includes observation date, percent emergence and dry shoot weight. The means and standard deviations for control(s) and treatment replicate measurements will be calculated and subjected to statistical analysis.

The control and solvent control data will be compared using a two-tailed t Test. If the data are similar, the control and solvent control values will be pooled for further analysis with the treatment data. If a significant difference is detected between the control and solvent control data, the treatment data will be compared to the solvent control data.

Mean percent emergence and dry shoot weight of the treated plants will be calculated as a percentage relative to the appropriate control data (e.g., percent inhibition).

4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

5.0 REPORTING

The raw data and final draft of the report will be reviewed by the Quality Assurance Unit and the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report will initially be submitted to the Study Sponsor for review. Upon acceptance by the Sponsor, three copies of the final report will be submitted. All reports will include, but will not be limited to, the following information:

- Springborn Smithers Laboratories report and project numbers and Sponsor study numbers (if any).
- Laboratory and site, the dates of testing and personnel involved in the study, i.e., Program Coordinator (if applicable), Study Director, Principal Investigator.
- Identification of the test substance which may include chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, water solubility, vapor pressure, degree of purity of test article (percent test chemical) (Sponsor supplied, if available).
- Information about the test plants: species and variety used, seed source (packager or supplier), seed lot, and germination percentage.
- Description of the test method or attached literature reference describing the method used.
- Conditions of testing:
 - a. Carriers, emulsifiers, solvents, and/or additives used and their concentrations.
 - b. Mean test temperature (\pm standard deviation) and range throughout the test period.
 - c. Photoperiod if conducted under light/dark conditions.
 - d. Relative humidity range throughout the test.
 - e. Method of test chemical introduction and concentrations.
 - f. Source and description of water used to prepare the water soluble fertilizer.
 - g. Number of replicates per concentration or control.
 - h. Characterization of the support medium (e.g., percent-organic matter, pH).
 - i. Method of assignment and positioning of seeds/seedlings.
- Number and percentage of seedlings that showed any adverse effect in the controls and treatments at the conclusion of the test.
- A description of the statistical procedures used.

- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.
- Location of raw data and report.

6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any. If necessary, amendments other than the one providing the information required by page one of this protocol, may initially be verbally authorized, followed by Springborn Smithers' written documentation. In such cases, the effective date of the amendment will be the date of verbal authorization.

7.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records and reports will comply with the Organization of Economic Co-operation and Development's (OECD) Good Laboratory Practices as set forth under the OECD Guidelines for the Testing of Chemicals.

8.0 REFERENCES

- Daniel, W. W. 1990. Applied Nonparametric Statistics, 2 ed. PWS-KENT Publishing Company: Boston, Massachusetts. 635 pp.
- Gulley, D.D., Boetler, A.M. and Bergman, H.L. 1996 Toxstat Release 3.5. University of Wyoming, Laramie, Wyoming.
- OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris, France. 41 pp.
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- U.S. EPA. 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants. PB83-153940, U. S. Environmental Protection Agency, Washington, DC.
- U.S. EPA. 1986. Hazard Evaluation Division. Standard Evaluation Procedure. Non-Target Plants: Seed Germination/Seedling Emergence/ Vegetative Vigor. EPA 540/9-86-132. U.S. EPA Washington, D.C.

U.S. EPA. 1994. Pesticide Reregistration Rejection Rate Analysis: Ecological Effects. EPA 738-R-94-035, U.S. Environmental Protection Agency, Washington, DC.

Weber, C.I. et al. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, second edition. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/489/001.

Zar, J.H. 1984. Biostatistical Analysis, 2 ed. Prentice Hall, Inc.: Englewood Cliffs, NJ, 718 pp.

**SPRINGBORN SMITHERS
LABORATORIES**

Massachusetts Research Center

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PROTOCOL AMENDMENT

Amendment No.: 1
Effective Date: 15 February 2007
Protocol Title: Seedling Emergence and Seedling Growth Test Following OECD Draft Guideline #208
Protocol Number: 101006/OECD/Emergence and Growth/6 species/BPA
Species: 6 species
Study Sponsor: American Chemistry Council
Test Substance: Bisphenol A
Springborn Study No.: 13761.6123

Amendment:**1. Page 1, Cover Page**

The following information is required to distinguish the materials used in testing and for analysis of the stock solutions at Springborn Smithers Laboratories. The test substance was a combination of radio-labeled [^{14}C] and non-radiolabeled [^{12}C] bisphenol A.

Test Substance: ^{14}C -bisphenol A	Lot # 151-126-200	Radiochemical Purity: 99.8%
Test Substance: ^{12}C -bisphenol A	Lot # B0070138	Purity: 99.62%

Analytical Standard used at Springborn Smithers Laboratories for stock solution confirmation:
 ^{12}C -bisphenol A Lot # B0070138 Purity: 99.62%

2. Page 4, Section 2.2.4 Irrigation

At the Study Sponsor's request, a sample of the well water to be used to irrigate the plants will be collected and analyzed for residual bisphenol A concentration. The same water source will be used to prepare the water-soluble fertilizer also to be used to irrigate the plants. The analysis will be performed by ABC Laboratories, Columbia, Missouri. Two additional water samples will be collected and held at Springborn Smithers Laboratories as archive samples in the event they are needed for analysis.

3. Page 5, Section 2.4.3 Seedling Observations

The protocol states that 14 days after $\geq 50\%$ emergence is determined in the control, the number of emerged plants will be recorded. To better characterize the emergence of seedlings in the study, seedling emergence will also be recorded on day 7 post-50% emergence in the control.

4. Page 6, Section 2.5 Analytical Methodology

The protocol states that three samples will be collected for confirmation of total ^{14}C -radioactive concentration of each dosing solution before use in the test. At the Study Sponsor's request, the total concentration of bisphenol A in each stock solution will also be measured by HPLC/UV analysis. Since the stock solutions will be measured by two different analytical techniques, only one sample will be analyzed from each stock solution by each analytical technique.

Page 1 of 2

Other Locations: 2900 Quakenbush Road, P.O. Box 620 • Snow Camp, North Carolina 27349 • Phone: (336) 376-0141
• Fax: (336) 376-0145
Seestrasse 21 • Horn, CH-9326, Switzerland • Phone: (41) 71 844-6970 • Fax: (41) 71 841-8630

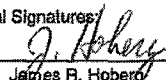
Letters, Reports and Protocols: Springborn Smithers Laboratories letters, reports and protocols are issued for the exclusive use of the clients to whom they are addressed. No quotations from reports or protocols or use of the Springborn Smithers Laboratories name is permitted except as expressly authorized in writing. Letters, reports and protocols apply only to the specific materials, products or processes tested, examined or surveyed and are not necessarily indicative of the qualities of apparently identical or similar materials, products or processes. The liability of Springborn Smithers Laboratories with respect to services rendered shall be limited to the amount of the consideration paid for such services and not include any consequential damages.

13761.6123
Page 2 of 2

The protocol states that three quality control (QC) samples will be prepared, stored if necessary, and analyzed with the set of study samples. To clarify, this statement refers to the radiometric analyses of soil samples only. Additionally, in place of QC samples, three SPEC-CHEC™ samples to indicate instrument performance, will be used to verify the efficiency of the analytical equipment prior to use. This revision was necessary since excess radiolabeled test substance was not available for QC samples.

None of the above changes will have a negative impact on the study.

Approval Signatures:

James R. Hoberg
Springborn Study Director

7/24/07

Date



Study Sponsor Representative

7/26/07

Date



Massachusetts Research Center
790 Main Street • Wareham, MA • 02571-1037 • Phone: (508) 295-2550 • Fax (508) 295-8107

PROTOCOL AMENDMENT

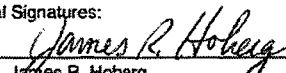
Amendment No.: 2
Effective Date: 6 August 2007
Protocol Title: Seedling Emergence and Seedling Growth Test Following OECD Guideline #208
Protocol Number: 101006/OECD/Emergence and Growth/6 species/BPA
Species: 6 species
Study Sponsor: American Chemistry Council
Test Substance: Bisphenol A
Springborn Study No.: 13761.6123

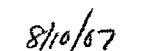
Amendment:**1. Page 1 and 2, Protocol Title**

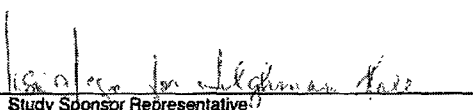
The protocol title is changed to: Seedling Emergence and Seedling Growth Tests Following OECD Guideline 208. The word "draft" was deleted from the title since the guideline was finalized on 19 July 2006 and is no longer a draft guideline.

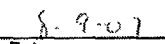
None of the above changes will have a negative impact on the study.

Approval Signatures:


James R. Hoberg
Springborn Study Director


Date


Study Sponsor Representative


Date

Page 1 of 1

Other Locations: 2900 Quakenbush Road, P.O. Box 620 • Snow Camp North Carolina 27349 • Phone: (336) 376-0141
• Fax: (336) 376-0145
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APPENDIX 2 - CERTIFICATE OF ANALYSIS

03/26/2007 01:42 FAX 703 741 5651

PSPC & ERSC

001

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Telephone 919 541-6000 • Fax 919 541-5985 • www.rti.org

SSL-108-53

**RTI INTERNATIONAL
COMPOUND ANALYSIS REPORT
BISPHENOL A**Analysis Date: October 11, 2006
Date of This Report: February 9, 2007RTI Project No.: 0209257.001
RTI Protocol No.: RTI-675-AN
RTI Notebook No.: 11341 pp.: 50-73Compound: Bisphenol A
CAS No.: 80-05-7
Formula: $C_{15}H_{16}O_2$
Formula Weight: 228.28
Vendor: Acros Organics
Vendor Lot No.: B0070138
Analytical Sample Log No.: 9176-38-01
Storage Conditions: Room temperature
Appearance: Opaque white granular solid**Purity Determination**

HPLC (UV at 210 nm): 99.62% of total integrated area

Component	Retention Time (min)	% of Total area
impurity A	5.4	< 0.01
impurity B	5.8	< 0.01
impurity C	6.8	< 0.01
Bisphenol A	8.0	99.62
impurity D	10.1	0.12
impurity E	13.6	0.01
impurity F	14.7	0.01
impurity G	22.0	0.01
impurity H	24.0	0.01
impurity I	25.2	< 0.01
impurity J	27.7	0.02
impurity K	28.9	0.01
impurity L	34.2	0.12
impurity M	35.9	0.06

Comment: Technical questions about this compound analysis should be directed to
Mr. Stephen D. Cooper at (919) 541-6595Verified by: *K.E. Amato* Date: 2/9/2007Approved by: *S.D. Cooper* Date: 2/9/2007

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APPENDIX 3 – PREPARATION OF STOCK SOLUTIONS

**Analytical Report
Moravek Biochemicals**

Report Title				
Preparation and Analysis of ^{14}C -Bisphenol A Stock Solutions for use in Ecotox Testing				
Reviewer		Signature	Date	
Robert Todd		<i>[Signature]</i>	3-1-7	
Reviewer		Signature	Date	
Rick Horn		<i>[Signature]</i>	3-1-07	
Location	Department	File	Date Issued	Page Count
Brea, California	Quality Control	3	03/01/2007	66
Test Material(s)		Source	Lot Number	
^{14}C -Bisphenol A		Moravek Biochemicals	151-126-200	
^{12}C -Bisphenol A		American Plastics Council	B0070138	
Date Samples Prepared		January 3-4, 2007		
Analysis Date(s)		January 4-5, 2007		
Analysis Method		UV Spectroscopy and LSC		
Method Reference		See below		
Summary:				
<p>This report describes the preparation and analytical characterization of ^{14}C-bisphenol A stock solutions to be used for ecotoxicological testing at ECT and Springborn Laboratories. Varying amounts of ^{12}C-bisphenol A (received from the American Plastics Council) were mixed with ^{14}C-bisphenol A (Moravek Biochemicals), dissolved in acetone, and dispensed into serum bottles. A total of 34 individual dosing solutions were prepared according to the specifications outlined in Table 1.</p> <p>Samples of each dosing solution were analyzed by UV spectroscopy to measure the total concentration of bisphenol A in the sample. A summary of the UV spectral analysis is summarized in Table 3. Samples from one dosing solution were also analyzed by liquid scintillation counting in order to determine the total radioactivity in the solution, and the specific activity of the test material in that solution.</p>				

Test Materials

¹⁴C-Bisphenol A, [ring-¹⁴C(U)]-
Lot Number: 151-126-200
Specific Activity: 200 mCi/mmol
Concentration: 1.0 mCi/ml; 1.14 mg/ml
Packaged in: Acetone
Date of Analysis: December 6, 2006
Radiochemical Purity: 99.8%
Column: Supelco Discovery C18 4.6 x 250mm
Flow Rate: 1.0 ml/min.
Mobile Phase: A: 0.01% TFA in water
 B: 0.01% TFA in acetonitrile
 0-5 min. 5% B
 5-30 min. 5-100% B
 Hold to 40 min.

Details of the description and characterization of the ¹⁴C material are provided in Appendix 1.

¹²C-Bisphenol A

Lot Number: B0070138
Physical State: Solid
Purity: 99.62%
Date of Analysis: October 11, 2006

Details of the description and characterization of the ¹²C material are provided in Appendix 2.

Methods for Preparation of Dosing Solutions

Using a Mettler AT261 DeltaRange balance (S/N L62353), each sample vial was prepared according to the Dosing Solution Chart summarized in Table 1. The vials were consecutively numbered from 1 to 34. Samples for the Collembola Test (ECT Lab) consist of vials labeled one through ten. The Enchytraeus Test (ECT Lab) samples consist of vials identified 11 through 22, and the Plant Test (Springborn Lab) samples consist of vials identified 23 through 34. Note that due to test sample breakage at the receiving laboratory, vial 34 had to be re-prepared, and was designated vial 34R.

The designated amount of ¹²C-bisphenol A (Lot # B0070138) was placed into a tared vial, and the appropriate amount of acetone (EMD HPLC Grade Lot #46195) was added. The final step for preparing the dosing solutions was to add the ¹⁴C-labeled bisphenol A (Lot# 151-262-200) in acetone at a concentration of 1 mCi/mL with a specific activity of 200 mCi/mmol. The additions were made using a calibrated Eppendorf pipetor. Vials 1 to 22 each received 4 uCi of the ¹⁴C-labeled material. Samples 28, 34, and 34R each received 60 uCi of the ¹⁴C-labeled material. Samples 23 to 27 and 29 to 33 each received 120 uCi ¹⁴C-labeled material.

Note that for sample vials 11 and 12, the quantity of ¹²C-bisphenol A was too small to be achieved by individual weighing. To prepare these samples, a concentrated solution of

19.544 mg ^{12}C -bisphenol A in 50 mL of acetone was prepared. A portion (0.5 mL) of this concentrated solution was added to the sample vials. The appropriate volumes of acetone and ^{14}C -bisphenol A solutions were then added.

Table 1. Preparation of Dosing Solutions: Nominal and Actual Composition

ECT Solutions Sample Vial ID #	Nominal Composition of Solution			Measured Composition of Solution		
	Amount of ¹² C-BPA (mg)	Amount of ¹⁴ C-BPA (μ Ci)	Volume Acetone (mL)	Nominal Total BPA Concentration (mg/mL)	Actual Amount ¹² C-BPA (mg)	Actual Amount ¹⁴ C-BPA (μ L) ¹
1	12.49544	4	5	2.5	12.49	4
2	12.49544	4	5	2.5	12.49	4
3	24.99544	4	5	5.0	24.99	4
4	24.99544	4	5	5.0	24.99	4
5	49.99544	4	5	10.0	49.99	4
6	49.99544	4	5	10.0	49.99	4
7	99.99544	4	5	20.0	99.99	4
8	99.99544	4	5	20.0	99.99	4
9	199.99544	4	5	40.0	199.99	4
10	199.99544	4	5	40.0	199.99	4
11	0.19544	4	5	0.04	0.195	4
12	0.19544	4	5	0.04	0.195	4
13	1.89544	4	5	0.38	1.89	4
14	1.89544	4	5	0.38	1.89	4
15	3.41544	4	5	0.68	3.41	4
16	3.41544	4	5	0.68	3.41	4
17	6.17544	4	5	1.24	6.17	4
18	6.17544	4	5	1.24	6.17	4
19	11.11544	4	5	2.22	11.11	4
20	11.11544	4	5	2.22	11.11	4
21	19.99544	4	5	4.0	19.99	4
22	19.99544	4	5	4.0	19.99	4

Note 1: ¹⁴C-Bisphenol A added in an acetone solution at a concentration of 1 mCi/mL with a specific activity of 200 mCi/mmolNote 2: 4 μ Ci of ¹⁴C-bisphenol is equivalent to 0.00457 mg based on a specific activity of 200 mCi/mmol and m.w. of 228.3

Table 1 (cont'd). Preparation of Dosing Solutions: Nominal and Actual Composition

Springborn Solutions	Sample Vial ID #	Nominal Composition of Solution				Measured Composition of Solution			
		Amount of ¹² C BPA (mg)	Amount of ¹⁴ C BPA (uCi)	Volume Acetone (mL)	Nominal Total BPA Concentration (mg/mL)	Actual Amount ¹² C-BPA (mg)	Actual Amount ¹⁴ C BPA (uL) ¹	Actual Volume Acetone Dispensed (mL)	Calculated Total BPA Concentration (mg/mL) ²
	23	11999.8632	120	50	240	11999.86	120	49.88	240.0
	24	11999.8632	120	50	240	11999.86	120	49.88	240.0
	25	11999.8632	120	50	240	11999.86	120	49.88	240.0
	26	11999.8632	120	50	240	11999.86	120	49.88	240.0
	27	11999.8632	120	50	240	11999.86	120	49.88	240.0
	28	5999.9316	60	50	120	5999.93	60	49.94	120.0
	29	1799.8632	120	50	36	1799.86	120	49.88	36.0
	30	1799.8632	120	50	36	1799.86	120	49.88	36.0
	31	1799.8632	120	50	36	1799.86	120	49.88	36.0
	32	1799.8632	120	50	36	1799.86	120	49.88	36.0
	33	1799.8632	120	50	36	1799.86	120	49.88	36.0
	34	899.9316	60	50	18	899.93	60	49.94	18.0
	34R	899.9316	60	50	18	899.93	60	49.94	18.0

Note 1: ¹⁴C-Bisphenol A added in an acetone solution at a concentration of 1 mCi/mL with a specific activity of 200 mCi/mmol
 120 uCi of ¹⁴C-bisphenol is equivalent to 0.137 mg (60 uCi equals 0.0684 mg) based on a specific activity of 200 mCi/mmol and m.w. of 228.3

Analysis of Dosing Solutions

Instrument	Dual Beam Shimadzu UV 1700 PharmaSpec Spectrophotometer
Cuvettes	3 mL, 1 cm pathlength quartz cuvette
Solvent	Ethanol, Rossville Gold Shield grade, 200 pf

Determination of Extinction Coefficient

Prior to the analysis of the sample vials, the extinction coefficient for bisphenol A was determined by spectrophotometric analysis of known standards of ^{12}C -bisphenol A prepared in ethanol. A concentrated stock solution of ^{12}C -bisphenol A was prepared by dissolving 10 mg of material in 10 mL of acetone, resulting in a 1 mg/mL solution. Varying amounts were added to 3 mL of ethanol in a cuvette. The UV spectrum of the sample was determined over the range from 200 nm to 400 nm.

Results of the analysis are summarized in Table 2. Based on linear regression of the response curve, the extinction coefficient at 228 nm was determined to be $14.683 \text{ mM}^{-1} \text{ cm}^{-1}$.

Analysis of ^{14}C -Bisphenol A Sample Vials

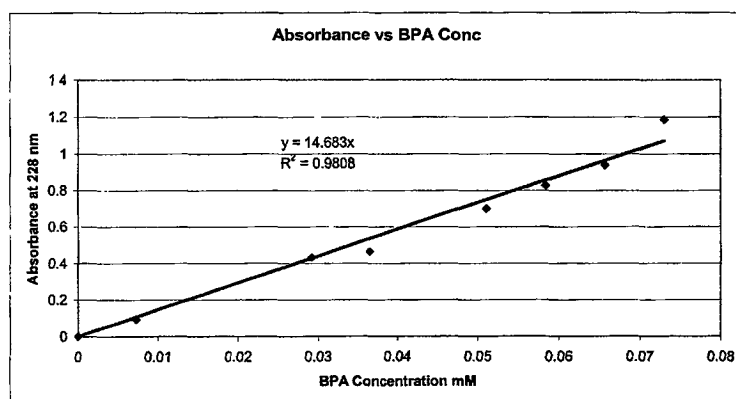
To prepare dilutions of the dosing solutions for spectrophotometric analysis, varying portions of each dosing solution were diluted into an appropriate volume of ethanol as described in Table 3. A 3 mL aliquot of the dilution was transferred to the cuvette, and the UV spectrum was recorded over the range from 200 nm to 400 nm. The absorbance at 228 nm was determined and used to calculate the total bisphenol A concentration for each dosing solution.

Identical reference solutions were prepared by adding the same volume of acetone to ethanol that was used to create each aliquot for ^{14}C -Bisphenol A.

Results of the spectrophotometric analysis are summarized in Table 3. There was general agreement between the measured and nominal concentrations for each of the dosing solutions; the percentages of measured to nominal concentrations ranged from 93.1 to 170.9%.

Table 2. Determination of Extinction Coefficient for ^{12}C -Bisphenol in Ethanol

Mass BPA ug	Vol EtOH mL	Concentration mM	Absorbance 228 nm	Extinction Coefficient mM-1cm-1
0	0	0	0	
5	3	0.0073	0.095	13.013
20	3	0.0292	0.433	14.828
25	3	0.0365	0.465	12.739
35	3	0.0511	0.700	13.698
40	3	0.0584	0.828	14.177
45	3	0.0657	0.940	14.307
50	3	0.0730	1.188	16.273



Equation 1. Absorbance = Extinction Coefficient (M-1cm-1) * Pathlength (cm) * Concentration (M)

Table 3. Results of Spectrophotometric Analysis of 14C-Bisphenol Dosing Solutions.

Table 3. Results of Spectrophotometric Analysis of 14C-Bisphenol Dosing Solutions

Dose Solution Sample ID #	Nominal [BPA] mg/mL	Vol Sample μ L	Vol EtOH μ L	Dilution Factor	Absorbance 228 nm	[BPA] in Dilution mg/L	[BPA] in Dose Solution mg/mL	% Nominal
1	2.5	5	1000	200	0.968	15.05	3.01	120.4
2	2.5	5	1000	200	0.871	13.54	2.71	108.3
3	5	5	1000	200	1.504	23.39	4.68	93.5
4	5	5	1000	200	1.498	23.29	4.66	93.2
5	10	5	4000	800	0.951	14.79	11.83	118.3
6	10	5	4000	800	0.934	14.52	11.62	116.2
7	20	5	8000	1600	0.881	13.70	21.92	109.6
8	20	5	8000	1600	1.026	15.95	25.52	127.6
9	40	5	16000	3200	0.938	13.03	41.70	104.2
10	40	5	16000	3200	0.884	13.74	43.98	110.0
11	0.04	300	1000	3.33	0.816	12.69	0.04	105.7
12	0.04	300	1000	3.33	0.838	13.03	0.04	108.6
13	0.38	5	1000	200	0.176	2.74	0.55	144.0
14	0.38	5	1000	200	0.209	3.25	0.65	171.0
15	0.68	5	1000	200	0.29	4.51	0.90	132.6
16	0.68	5	1000	200	0.313	4.87	0.97	143.1
17	1.24	5	1000	200	0.458	7.12	1.42	114.9
18	1.24	5	1000	200	0.513	7.98	1.60	128.7
19	2.22	5	1000	200	0.795	12.36	2.47	111.4
20	2.22	5	1000	200	0.839	13.05	2.61	117.5
21	4	5	1000	200	1.251	19.45	3.89	97.3
22	4	5	1000	200	1.361	21.16	4.23	105.8

Eq 1. [BPA] in Dilution (mg/L) = (Absorbance at 228 nm / Extinction Coefficient of 14.683 mM⁻¹cm⁻¹ * 1 cm path) * 228 mg/mmol

Eq 2. [BPA] in Dose Solution = ([BPA] Conc in Dilution (mg/L) * Dilution Factor) / 1000 (mL/L)

Table 3 (cont'd). Results of Spectrophotometric Analysis of 14C-Bisphenol Dosing Solutions.

Dose Solution Sample ID #	Nominal [BPA] mg/mL	Vol Sample uL	Vol EtOH uL	Dilution Factor	Absorbance 228 nm	[BPA] in Dilution mg/L	[BPA] in Dose Solution mg/mL	% Nominal
23	240	1	15000	15000	1.029	16.00	239.99	100.0
24	240	1	15000	15000	1.01	15.70	235.56	98.2
25	240	1	15000	15000	1.056	16.42	246.29	102.6
26	240	1	15000	15000	1.054	16.39	245.82	102.4
27	240	1	15000	15000	1.054	16.39	245.82	102.4
28	120	1	15000	15000	0.585	9.10	136.44	113.7
29	36	5	15000	3000	0.857	13.33	39.98	111.0
30	36	5	15000	3000	0.844	13.12	39.37	109.4
31	36	5	15000	3000	0.904	14.06	42.17	117.1
32	36	5	15000	3000	0.811	12.61	37.83	105.1
33	36	5	15000	3000	0.853	13.26	39.79	110.5
34	18	5	15000	3000	0.433	6.73	20.20	112.2
34R	18	5	15000	3000	0.431	6.70	20.10	111.7

Eq 1. [BPA] in Dilution (mg/L) = (Absorbance at 228 nm / Extinction Coefficient of 14,683 mM⁻¹cm⁻¹ * 1 cm path) * x 228 mg/mmol

Eq 2. [BPA] in Dose Solution = ([BPA] Conc in Dilution (mg/L) x Dilution Factor) / 1000 (mL/L)

Note 1. Documentation of Extinction Coefficient Determination provided in Table 2.

Note 2. Raw data for spectrophotometric analysis provided in Appendix 3.

Specific Activity Determination for Sample Vial #34R

In addition to the spectrophotometric analysis of dosing solution #34R, additional analyses were conducted to determine the specific activity of the sample. Triplicate portions (5 uL) of dosing solution #34R were transferred to 5 mL of scintillation cocktail (MP Biomedical Econolume Lot #70491) and analyzed using a Packard Tri-carb 2500 TR model B2500 (S/N 405300) liquid scintillation counter.

The instrument conditions were as follows:
14C window 12.0 – 156 keV
Efficiency is determined by internal standard.

Results for the radioactivity analysis were as follows:

LSC Sample #	14C DPM
1	14523
2	15062
3	14673
Mean	14752

Based on the mean, the total radioactivity in 5 uL of dosing solution was 0.00665 uCi. Hence, the dosing solution with a volume of 50 mL contains 66.5 uCi or 0.0665 mCi of radioactivity and is in agreement with the composition described in Table 1.

The measured total bisphenol A concentration in dosing solution #34R was 20.09 mg/mL. Based on a total volume of 50 mL and a molecular weight of 228, the solution contains 1004.5 mg or 4.4 mmole of bisphenol A.

The specific activity of the ^{14}C bisphenol A in dosing solution #34R is

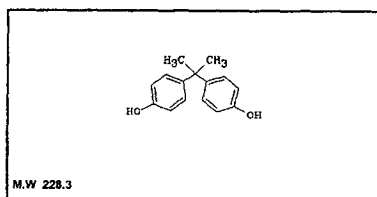
$$0.0665 \text{ mCi} / 4.4 \text{ mmol} = 0.015 \text{ mCi/mmol}.$$

Appendix 1. Analysis of 14C Material



Product Data Sheet

MC-2061

Bisphenol A, [ring-¹⁴C(U)]-

Lot #: 151-126-200

Specific Activity: 200 mCi/mmol

Concentration: 1.0mCi/ml; 1.14mg/ml

Packaged in: Acetone

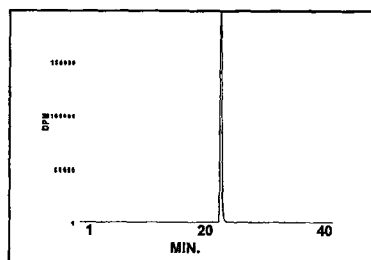
Date of Analysis: December 6, 2006

Radiochemical Purity: 99.8%

Column: Supelco Discovery C18 4.6 x 250mm

Flow Rate: 1.0 ml/min.

Mobile Phase: A: 0.01% TFA in water
B: 0.01% TFA in acetonitrile
0-5min 5%B, 5-30min 5-100%B
Hold to 40min



HPLC ANALYSIS LOT 151-126-200

12/06/06 08:27:19 CH= B PS= 1
FILE 2 METHOD 0 RUN 466 INDEX 466

PEAK#	AREA	RT	AREA BC
1	99.823	22.16	44546618.02
2	0.177	23.68	79078.03
TOTAL	100		446256.96

Stability and Storage Recommendation: Store at -20°C.

Product Warranty: Stated on the reverse side of this Product Data Sheet.

Caution: Not For Use In Humans Or Clinical Diagnosis. This product is intended for investigational or manufacturing use only. It is pharmaceutically unrefined and is not intended for use in humans. Responsibility for its use in humans, as a diagnostic reagent, and compliance with federal laws rests solely with the purchaser.

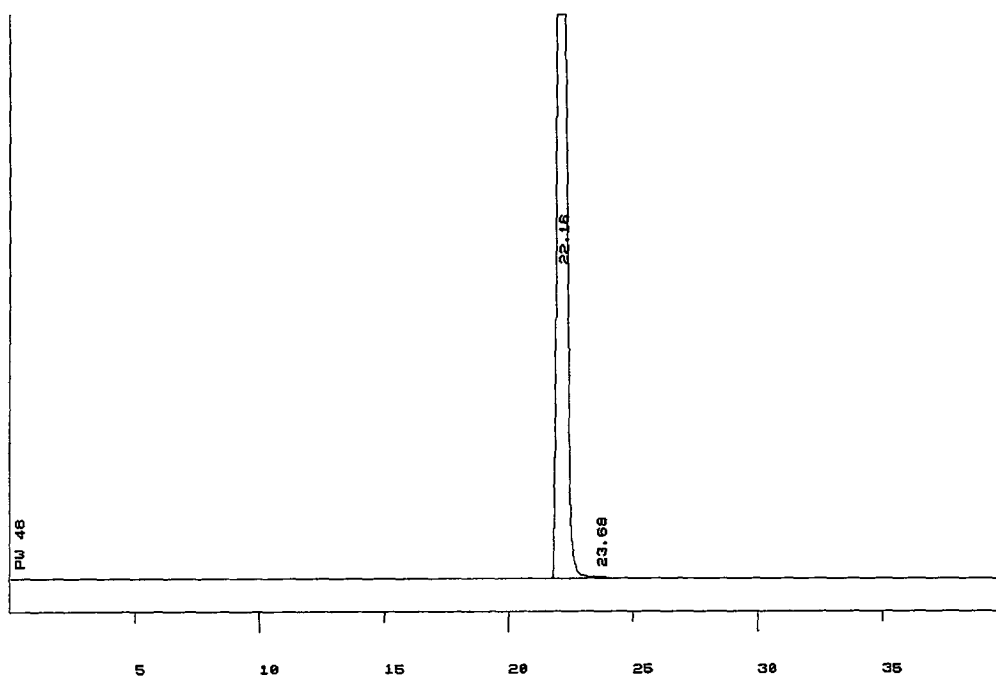
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FROM /4.A/

DISK FILE: INT46102.MDD

PEAK WIDTH: 6

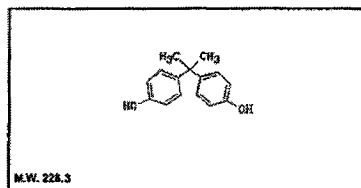
ATTENUATION: 512.0

MC2061, BISPHENOL A, (RING-14C); 151-126-200; PROFILE OF RADIOCHEM INJ; SEE
ATTACHED DATA SHEET FOR HPLC ANALYTICAL SYSTEM



Product Data Sheet

MC-2061

Bisphenol A, [ring¹⁴C(U)]-

Lot #: 151-126-200

Specific Activity: 200 mCi/mmol

Concentration: 1.0mCi/ml; 1.14mg/ml

Packaged in: Acetone

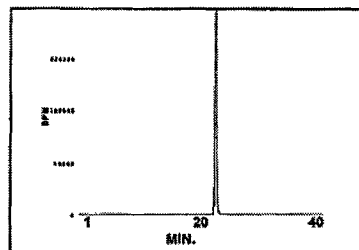
Date of Analysis: December 6, 2006

Radiochemical Purity: 99.8%

Column: Supelco Discovery C18 4.6 x 250mm

Flow Rate: 1.0 ml/min.

Mobile Phase: A: 0.01% TFA in water
B: 0.01% TFA in acetonitrile
0-5min 5%B, 5-30min 5-100%B
Hold to 40min



HPLC ANALYSIS LOT 151-126-200

12/06/06 08:27:19 CH= B PS= 1
FILE 2 METHOD 0 RUN 486 INDEX 486

PEAK#	AREA%	RT	AREA	BC
1	99.823	23.16	44546618.02	
2	0.177	23.68	79078.03	
TOTAL	100.		446256.06	

Stability and Storage Recommendation: Store at -20°C.

Product Warranty: Stated on the reverse side of this Product Data Sheet.

Caution: Not For Use In Humans Or Clinical Diagnosis. This product is intended for investigational or manufacturing use only. It is pharmaceutically unrefined and is not intended for use in humans. Responsibility for its use in humans, as a diagnostic reagent, and compliance with federal laws rests solely with the purchaser.

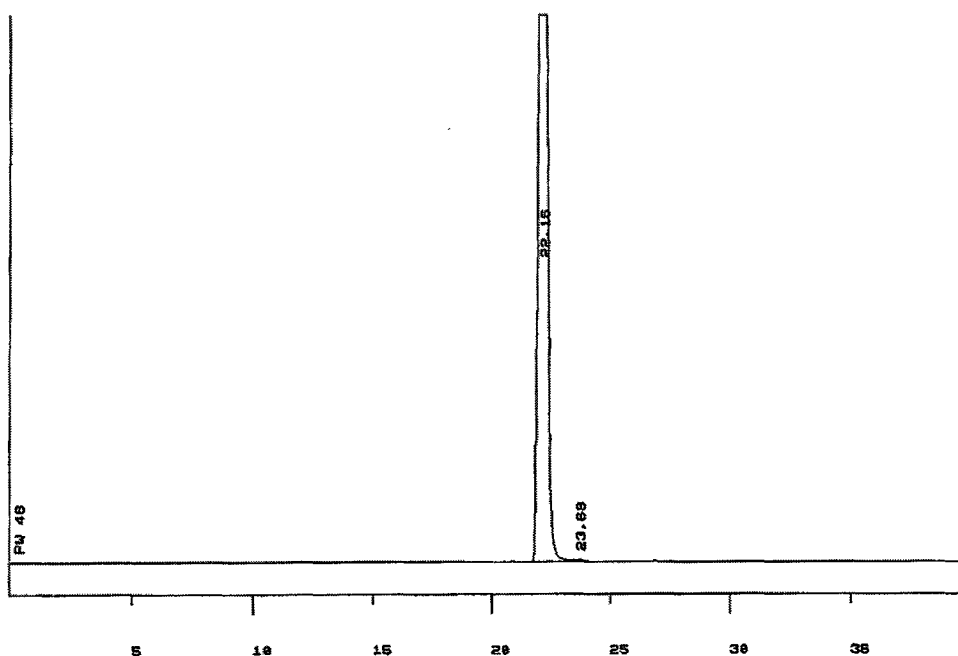
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FROM /4.A/

DISK FILE: INT46102.MOD

PEAK WIDTH: 6

ATTENUATION: 512.0

MC2061, BISPHENOL A, IRING-1403; 151-126-200; PROFILE OF RADIOCHEM INJ; SEE
ATTACHED DATA SHEET FOR HPLC ANALYTICAL SYSTEM

DISK FILE: INT46102.MOD FROM / 4.A/ INJECTED AT: 12/06/06 08:27:19

FILE	1	METHOD	0	RUN	1	INDEX	1	CH=	PS=	1
PEAK#		AREA%		RT		AREA	BC			
1		99.823		22.16		44546618	02			
2		0.177		23.68		79078	03			
TOTAL		100.				44625696				

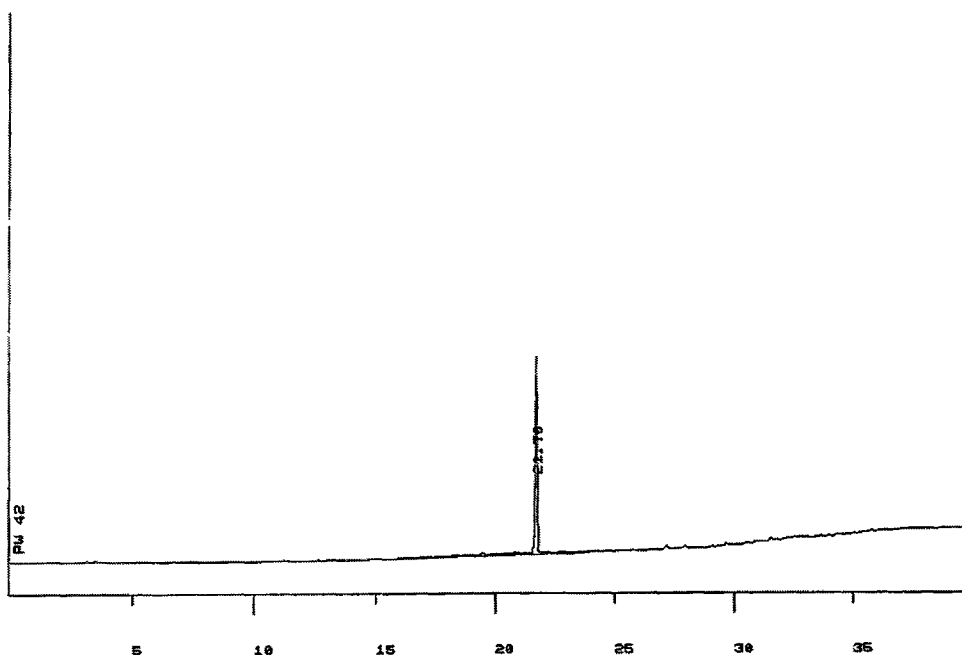
INJECTED AT 12/06/86 08:27:21

FROM /4.B/

DISK FILE: INT48741.MOD

PEAK WIDTH: 6

ATTENUATION: 512.0

MC2051, BISPHENOL A, IRING-1401; 151-126-200; UV TRACE OF RADIOCHEM INJ; SEE
ATTACHED DATA SHEET FOR HPLC ANALYTICAL SYSTEM

DISK FILE: INT4B741.MOD FROM / 4.B/ INJECTED AT: 12/06/06 08:27:21

FILE	1	METHOD	0	RUN	1	INDEX	1	CH=	PS=	1
PEAK#		AREA%		RT		AREA	BC			
1		100.		21.7		2750342	03			
TOTAL		100.				2750342				

INJECTED AT 12/06/06 09:22:06

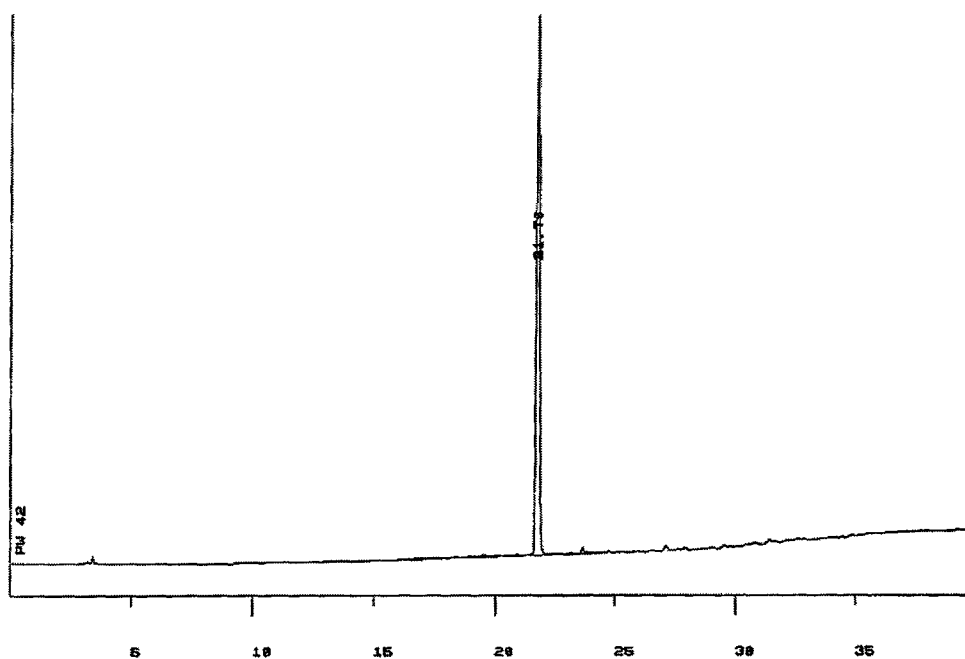
FROM /4.B/

DISK FILE: INY4B742.MDD

PEAK WIDTH: 6

ATTENUATION: 512.0

MC2061, BISPENOL A, [RING-14C]; LOT# 151-126-200; UV PROFILE OF STD; SEE
ATTACHED DATA SHEET FOR HPLC ANALYTICAL SYSTEM



DISK FILE: INT4B742.MOD FROM / 4.B/ INJECTED AT: 12/06/06 09:22:05

FILE	1	METHOD	0	RUN	1	INDEX	1	CH=	PS=	1
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1		100.		21.7		19983872	03			
TOTAL		100.				19983872				

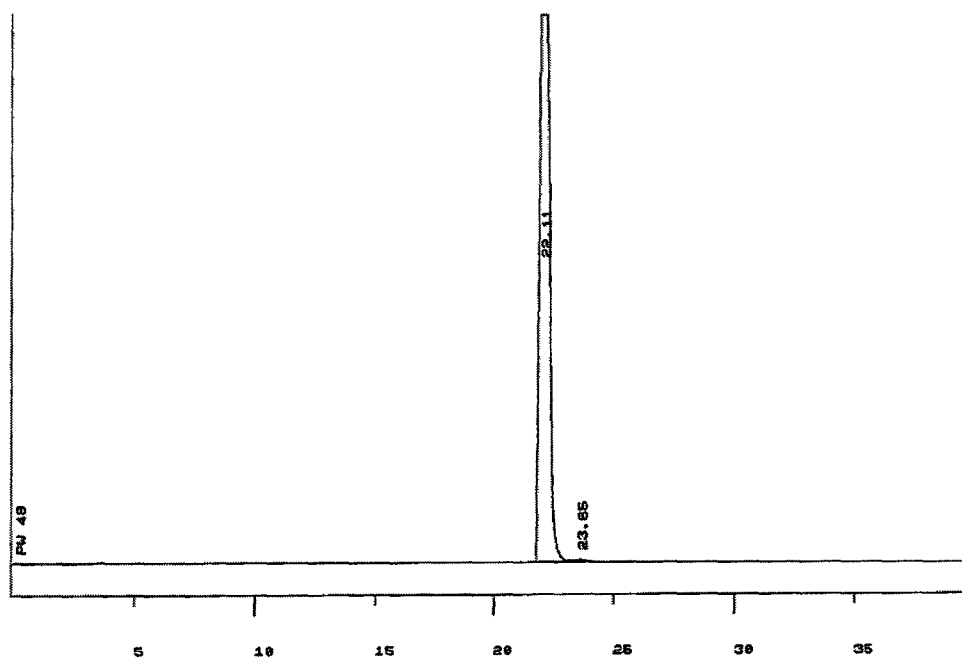
INJECTED AT 12/05/06 16:07:29

FROM /4.A/

DISK FILE: INT46101.MOD

PEAK WIDTH: 6

ATTENUATION: 512.0

MC2061, BISPHENOL A, (RING-14C); LOT# 151-126-200; RADIOCHEM PROFILE OF COINJ W/
INT# 48740; SEE ATTACHED DATA SHEET FOR HPLC ANALYTICAL SYSTEM

DISK FILE: INT46101.MOD FROM / 4.A/ INJECTED AT: 12/05/06 16:07:29

FILE 1 METHOD 0 RUN 1 INDEX 1 CH= PS= 1

PEAK#	AREA%	RT	AREA BC
1	99.674	22.11	42786253 02
2	0.326	23.65	139776 03
TOTAL	100.		42926029

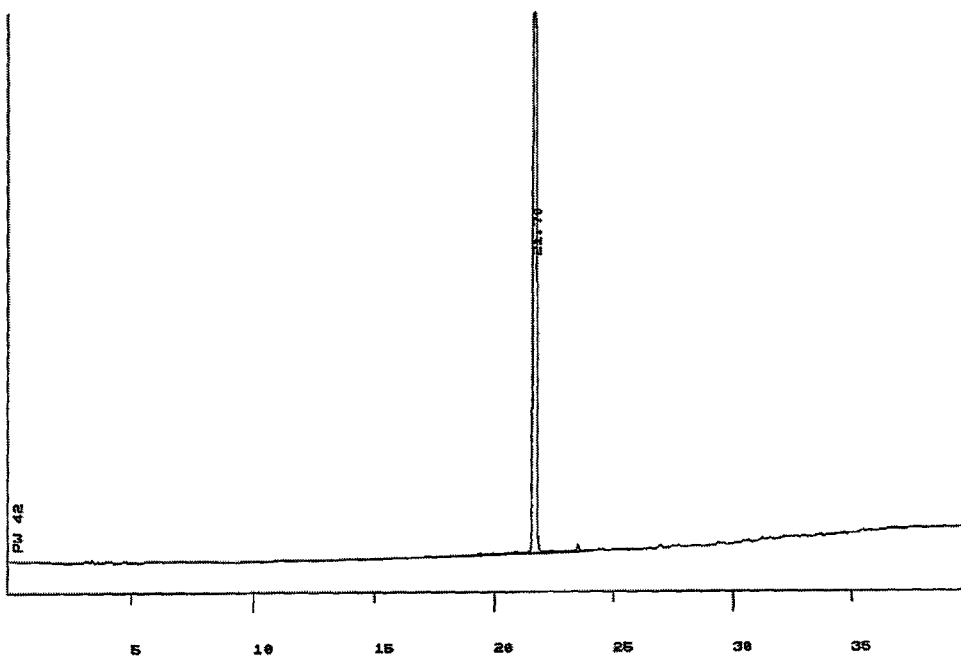
INJECTED AT 12/05/00 16:07:31

FROM /4.5/

DISK FILE: INT4B740.M00

PEAK WIDTH: 6

ATTENUATION: 512.0

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46101; SEE ATTACHED DATA SHEET FOR HPLC ANALYTICAL SYSTEM

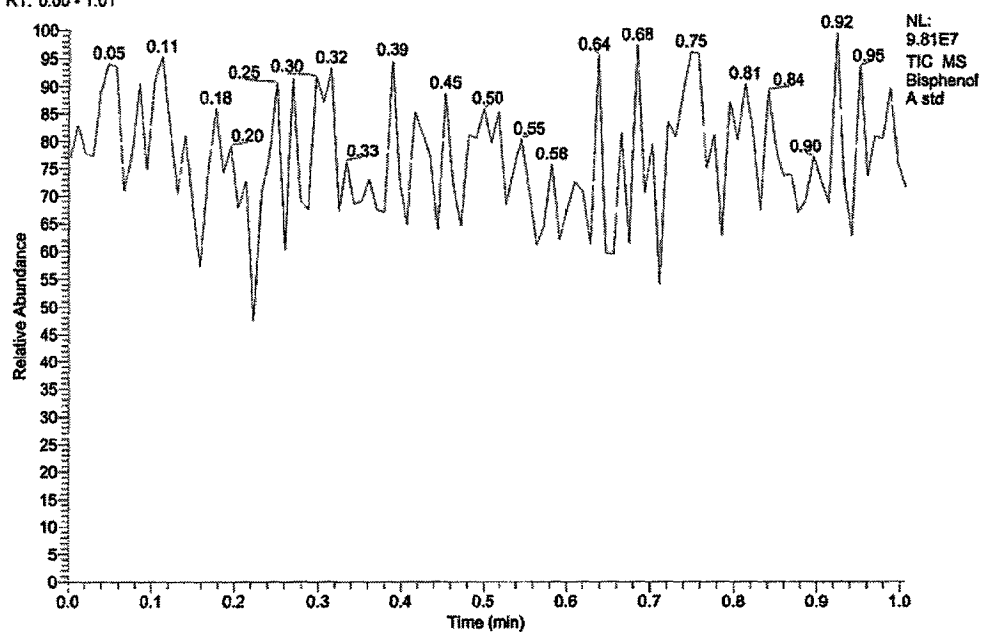
DISK FILE: INT4B740.MOD FROM / 4.B/ INJECTED AT: 12/05/06 16:07:31

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1		100.		21.7		19393702	03			
TOTAL		100.				19393702				

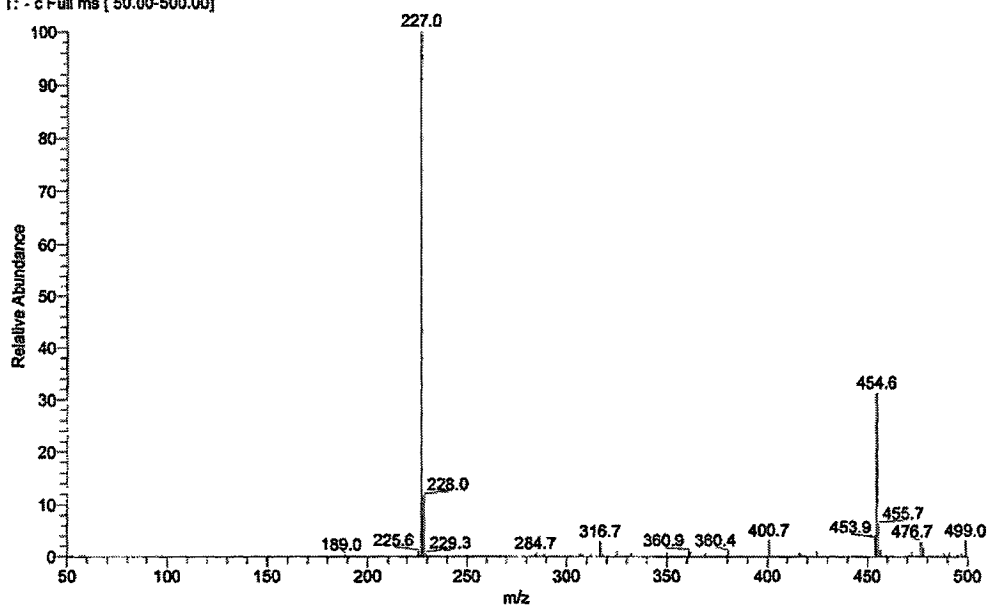
C:\Xcalibur\data\Bisphenol A std
Continuous infusion via syringe pump
RT: 0.00 - 1.01

12/2/2006 9:32:37 AM

Bisphenol A std



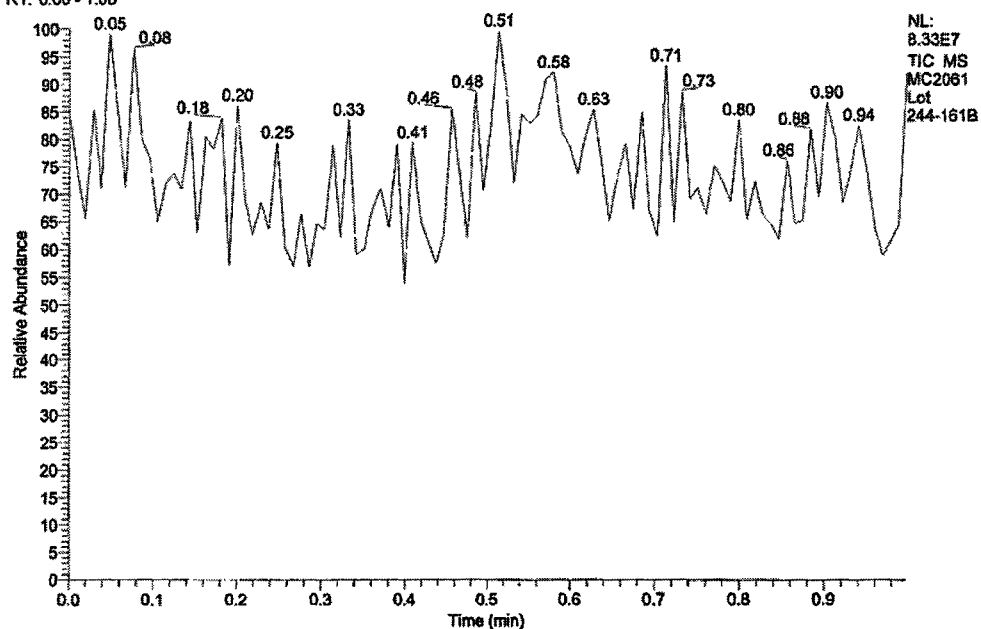
Bisphenol A std #1 RT: 0.00 AV: 1 NL: 4.02E7
T: - c Full ms [50.00-500.00]



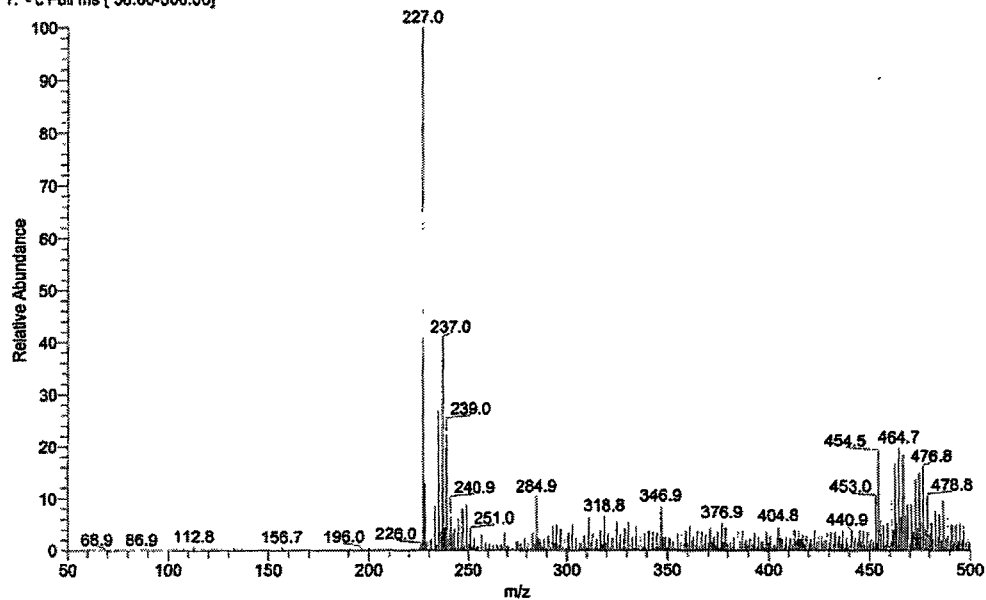
C:\Xcalibur\data\MC2061 Lot 244-161B
Continuous infusion via syringe pump
RT: 0.00 - 1.00

12/2/2006 9:40:15 AM

MC2061 Lot 244-161B



MC2061 Lot 244-161B #1-106 RT: 0.00-1.00 AV: 106 NL: 5.89E6
T: - c Full ms [50.00-500.00]



Appendix 2. Certificate of Analysis for 12C BPA

Fax from : 783 741 5651

82-26-87 07:33 Pg: 1



3040 Cornwallis Road • PO Box 12194 • Research Triangle Park, NC 27709-2194 • USA
 Telephone 919 541-5000 • Fax 919 541-5985 • www.rti.org

**RTI INTERNATIONAL
 COMPOUND ANALYSIS REPORT
 BISPHENOL A**

Analysis Date: October 11, 2006
 Date of This Report: February 9, 2007

RTI Project No.: 0209257.001
 RTI Protocol No.: RTI-676-AN
 RTI Notebook No.: 11341 pp.: 50-73

Compound: Bisphenol A
 CAS No.: 80-05-7
 Formula: $C_{15}H_{16}O_2$
 Formula Weight: 228.28
 Vendor: Acros Organics
 Vendor Lot No.: B0070138
 Analytical Sample Log No.: 9176-36-01
 Storage Conditions: Room temperature
 Appearance: Opaque white granular solid

Post-It® Fax Note	7671	Date	2/24/07	# of Pages	1
To	Rick Horn	From	Steve Hentzel		
Co./Dept.	Moravet	Co.	ACC		
Phone #	714 990 2018	Phone #	703 741 5588		
Fax #	714 990 1824	Fax #	703 741 6588		

Purity Determination

HPLC (UV at 210 nm): 99.62% of total integrated area

Component	Retention Time (min)	% of Total area
impurity A	5.4	< 0.01
impurity B	5.6	< 0.01
impurity C	6.8	< 0.01
Bisphenol A	8.0	99.62
impurity D	10.1	0.12
impurity E	13.6	0.01
impurity F	14.7	0.01
impurity G	22.0	0.01
impurity H	24.0	0.01
impurity I	25.2	< 0.01
impurity J	27.7	0.02
impurity K	28.9	0.01
impurity L	34.2	0.12
impurity M	35.9	0.06

Comment: Technical questions about this compound analysis should be directed to Mr. Stephen D. Cooper at (919) 541-6595.

Verified by: *H.E. Amato* Date: 2/9/2007

Approved by: *S.D. Cooper* Date: 2/9/2007

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Appendix 3. Raw Data for Analysis of Dosing Solutions

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**SPRINGBORN SMITHERS
LABORATORIES**

Study Title

Bisphenol A – Determination of Effects on
Seedling Emergence and Seedling Growth
(Definitive Tests)

Data Requirement

OECD Guideline Number 208

Author

James R. Hoberg

Study Completed On

10 August 2007

Submitted to

American Chemistry Council
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Arlington, Virginia 22209

Performing Laboratory

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Laboratory Project ID

Springborn Smithers Study No. 13761.6124

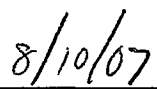
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for "**Bisphenol A – Determination of Effects on Seedling Emergence and Seedling Growth (Definitive Tests)**" were produced and compiled in accordance with all pertinent OECD Good Laboratory Practice Regulations (OECD, 1998) with the following exceptions: routine soil and water screening analyses were conducted at Agvise Laboratories, Northwood, North Dakota, and GeoLabs, Inc., Braintree, Massachusetts, respectively, using standard U.S. EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

SPRINGBORN SMITHERS LABORATORIES



James R. Hoberg
Study Director




Date

QUALITY ASSURANCE UNIT STATEMENT

The study conduct, raw data and report for "**Bisphenol A – Determination of Effects on Seedling Emergence and Seedling Growth (Definitive Tests)**" were inspected by the Quality Assurance Unit at Springborn Smithers Laboratories to determine adherence with the study protocol and laboratory standard operating procedures. Dates of study inspections, inspection types, and dates reported to the Study Director and to Management are given below.

<u>Inspection Date</u>	<u>Inspection Type</u>	<u>Reported to Study Director/Management</u>
5/2/07	Protocol review	5/2/07
5/21/07	Day 14, wheat observations	5/21/07
6/20-22/07	Data	6/21-22/07
7/13/07	Data	7/13/07
7/25-26/07	Draft report	7/26/07
8/8/07	Revised draft report	8/8/07
8/10/07	Final report	8/10/07

SPRINGBORN SMITHERS LABORATORIES



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8.10.07

Date

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The following Springborn Smithers personnel were responsible for the conduct of the work and reporting of the study results.

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SUMMARY

Bisphenol A - Determination of Effects on Seedling Emergence and Seedling Growth (Definitive Tests)

SPONSOR: American Chemistry Council

PROTOCOL TITLE: Seedling Emergence and Seedling Growth Test Following OECD Guideline #208," Springborn Smithers Laboratories Protocol No.: 041207/OECD/Emergence and Growth/6 species/BPA

SPRINGBORN SMITHERS STUDY NUMBER: 13761.6124

TEST SUBSTANCES: Bisphenol A, Lot No. B0070138, CAS No. 80-05-7, reported to have a purity of 99.62% was received from Research Triangle Institute on 26 October 2004.

TEST END POINTS: Percent emergence and dry shoot weight

APPLICATION OF TEST SUBSTANCE: Mixed into sandy loam

TEST SPECIES: Cabbage (*Brassica oleracea*)
Corn (*Zea mays*)
Oat (*Avena sativa*)
Soybean (*Glycine max*)
Tomato (*Lycopersicon esculentum*)
Wheat (*Triticum aestivum*)

EFFECT CRITERIA: Percent emergence and dry shoot weight, and treatment-related morphological abnormalities were determined for each species.

NOMINAL TEST CONCENTRATIONS¹: Cabbage: 20, 50, 130, 320 and 800 mg a.i./kg
Corn: 3.8, 10, 20, 50, 130 and 320 mg a.i./kg
Oat: 9.4, 19, 47, 120, 300 and 800 mg a.i./kg
Soybean: 20, 50, 130, 320 and 800 mg a.i./kg
Tomato: 4.0, 10, 20, 50, 130 and 320 mg a.i./kg
Wheat: 3.8, 9.4, 20, 47, 120 and 300 mg a.i./kg

¹ Nominal concentrations were selected based on the results of preliminary testing conducted under Springborn Smithers study number 13761.6123

MEASURED**CONCENTRATIONS:**

Each dosing stock used for application was analyzed by HPLC/UV methods. The measured concentrations indicated the dosing stock solutions closely approximated the desired nominal concentrations (See Table 3).

DATES OF DEFINITIVE TESTS

(including dry weights):

3 May to 4 June 2007

RESULTS:

Species	Percent Emergence Results (as mg a.i./kg) ^a			
	EC25 ^b	EC50 ^b	LOEC	NOEC
Cabbage	130 (83 – 180)	190 (120 – 230)	320	130
Corn	>320 NA ^c	>320 NA ^c	>320	320
Oat	>800 NA ^c	>800 NA ^c	>800	800
Soybean	650 (370 – 800)	>800 NA ^c	>800	800
Tomato	190 (160 – 210)	260 (230 – 300)	320	130
Wheat	>300 NA ^c	>300 NA ^c	>300	300

^a Results are based on nominal concentrations.

^b 95% confidence limits (in parentheses).

^c NA = Not Applicable. EC25 and EC50 values were empirically estimated, therefore, 95% confidence limits could not be calculated.

Dry Shoot Weight Results (as mg a.i./kg) ^a				
Species	EC25 ^b	EC50 ^b	LOEC	NOEC
Cabbage	82 (52 – 120)	>130 NA ^c	130	50
Corn	83 (14 – 180)	160 (80 – 280)	320	130
Oat	69 (57 – 81)	100 (87 – 130)	120	47
Soybean	220 (72 – 360)	460 (370 – 520)	800	320
Tomato	19 (9.8 – 32)	67 (52 – 79)	50	20
Wheat	120 (98 – 140)	200 (180 – 210)	120	47

^a Results are based on nominal concentrations.

^b 95% confidence limits (in parentheses).

^c NA = Not Applicable. EC50 value was empirically estimated, therefore, 95% confidence limits could not be calculated.

1.0 INTRODUCTION

The objective of this study was to determine the effects of bisphenol A on seedling emergence and early growth of six economically important, agricultural plant species. The definitive test concentrations were selected in consultation with the Study Sponsor based on previous testing at nominal concentrations of 150 and 1000 mg a.i./kg for each test species (SSL No. 13761.6123). The results of these definitive tests are based on nominal concentrations of bisphenol A and are reported as the 21-day EC25 and EC50 values for percent emergence and dry shoot weight. The 21-day No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) values for percent emergence and dry shoot weight were also determined for each species.

The study was initiated on 30 April 2007, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The tests were conducted from 3 May to 4 June 2007 at Springborn Smithers Laboratories (SSL) located in Wareham, Massachusetts. All raw data, the protocol and the original final report produced during this study are stored in Springborn Smithers' archives at the above location.

2.0 MATERIALS AND METHODS

2.1 Protocol

The procedures followed during this study are described in the Springborn Smithers Laboratories protocol entitled "Seedling Emergence and Seedling Growth Test Following OECD Guideline #208", Springborn Smithers Laboratories Protocol No.: 041207/OECD/Emergence and Growth/6 species/BPA (Appendix 1). The methods described in this protocol meet the requirements specified in the OECD Guideline for Testing of Chemicals #208, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD, 2006).

2.2 Test Substance

The test substance, bisphenol A, was received on 26 October 2004 from Research Triangle Institute, Research Triangle Park, North Carolina. The following information was provided:

Name:	Bisphenol A
Synonym:	BPA
Lot No.:	B0070138
CAS No.:	80-05-7
Purity:	99.62% (Appendix 2)
Date of Analysis:	11 October 2006 (most recent purity analysis for master Lot No. B0070138)
Expiration Date:	Stable, no expiration date assigned (per Study Sponsor)

Upon receipt at Springborn Smithers, the test substance (SSL No. 108-53) was stored at room temperature in the original container in a dark ventilated cabinet. This sample was used to prepare the exposure soil, analytical standards and quality control (QC) samples. Concentrations were adjusted for the purity of the test substance and are presented as active ingredient (a.i.).

Determination of stability and characterization, verification of the test substance identity, maintenance of records on the test substance, and archival of a sample of the test substance are the responsibility of the Study Sponsor.

2.3 Test Species

The plant species tested were three monocotyledons, corn (*Zea mays*), oats (*Avena sativa*) and wheat (*Triticum aestivum*), and three dicotyledons, cabbage (*Brassica oleracea*), soybean (*Glycine max*) and tomato (*Lycopersicon esculentum*). The seeds had not been pretreated with fungicides or insecticides. Seed variety, source, lot number, dates on which the seeds were packed and received, and the germination percentages for the seeds used during the seedling emergence tests are presented in Table 1. Upon receipt at Springborn Smithers, seeds were stored refrigerated at approximately 4 °C in the dark until test initiation.

2.4 Support Medium - Analyses and Characterization

Sandy loam collected from Fairhaven, Massachusetts (SSL Lot No. 063001) was purchased from Medeiros and Sons Trucking Company, Fairhaven, Massachusetts on 30 June 2001. The sandy loam was characterized by Agvise Labs, Northwood, North Dakota as containing 85% sand, 12% silt, 3% clay, with an organic carbon content of 1.1% (1.9% organic matter). A representative sample of the support medium was analyzed for the presence of pesticides, PCBs, and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were detected at concentrations that would compromise the results of this study (ASTM, 2002). The soil was heat-sterilized prior to use.

2.5 Exposure System

The exposure vessels consisted of polypropylene pots (Kord Products Ltd.). For cabbage, corn, soybean, oats and tomato, ten replicate pots were maintained for the control and each concentration tested. For wheat, five replicate pots were maintained for the control and each concentration tested. Each pot was 12-cm tall with a top diameter of 14 cm and a bottom diameter of 11.5 cm. The interior base was fitted with 20-cm diameter filter paper to retain the support medium and allow for plant uptake of the nutrient solution by sub-irrigation. The filter paper was added and then each pot was filled to a depth of 10 cm with 1.2 kg of support medium. Each pot was placed in a polypropylene saucer (Kord Products Ltd.) and received approximately 100 mL of well water via sub-irrigation.

The study was conducted in a greenhouse designed as follows: whenever natural light intensity fell below approximately 800 footcandles (8600 lux), sodium vapor lights supplemented natural light when necessary to maintain > 800 footcandles during the light period. The temperature was maintained between 24 to 42 °C, with heating or cooling from outside air as required.

The potential for air pollution within the greenhouse is believed to be minimal due to the rural location of the laboratory and the lack of other industrial businesses in the area. The greenhouse

is located in a relatively isolated section of the laboratory grounds, which reduces the possibility of air contamination from concurrent testing.

2.6 Well Water and Nutrient Solution

Well water was used to water the plants. Routine analyses for the presence of pesticides, PCBs and toxic metals were conducted periodically by GeoLabs, Inc., Braintree, Massachusetts, on representative samples of the well water provided. None of these compounds were detected at concentrations that would compromise the results of the study (ASTM, 2002) .

Additionally, the plants were sub-irrigated twice weekly with nutrient solution prepared from Peters 20-20-20 water soluble fertilizer (SSL No. 22102, supplied by Griffin Greenhouse Supplies) diluted to 200 mg/L with well water. Approximately 100 mL was provided to all pots by sub-irrigation twice weekly. All additional waterings were provided using well water.

2.7 Stock and Test Solution Preparation

A 192 mg a.i./mL primary stock solution was prepared by bringing 96.3654 g of test substance (95.9992 g as active ingredient) to a total volume of 500 mL with acetone. The resulting stock solution was observed to be clear and colorless with no visible undissolved test substance. This concentrated primary stock was used to prepare the dosing stock solutions as follows:

Stock Solution Used (mg a.i./mL)	Volume of Stock Solution Used (mL)	Brought to Total Volume with Acetone (mL)	Dosing Stock Concentration (mg a.i./mL)
192	100	250	76.8
192	10	50	38.4
192	40.63	250	31.2
192	4.06	50	15.6
192	15.63	250	12.0
192	1.56	50	6.0
192	6.25	250	4.8
192	2.5	200	2.4
192	0.313	50	1.2
192	0.50	100	0.96
192	0.125	50	0.48

All dosing stock solutions were observed to be clear and colorless with no visible undissolved test substance.

For the tests with cabbage, corn, oats, soybean and tomato, 47 to 50 mL of the appropriate stock solution was applied to 0.50 kg of silica sand. The difference in dosing volumes was due to a limited amount of stock solution available after analytical samples were collected. The treated sand was placed in a fume hood to allow the acetone to evaporate. Once dry, the treated sand was dispersed into 11.5 kg (dry weight) of sandy loam and mixed with a Hobart Mixer for 10 minutes to provide the desired nominal concentrations. For the test with wheat, a total of 6 kg of sandy loam was treated as described above. Solvent control soil was prepared prior to, and in the same manner as the treated soils (i.e., 50 mL acetone, applied to sand, evaporated and mixed in soil), but did not receive any test substance. The following table presents a summary of the dosing procedure used during this study:

Stock Conc. (mg a.i./mL)	Volume Stock Applied (mL)	Total Weight of Sand and Soil (kg)	Test Concentration (mg a.i./kg)	Test Species
0.48	47	6.0	3.8	Wheat
0.96	50	12	4.0	Tomato
0.96	47	12	3.8	Corn
1.2	47	6.0	9.4	Wheat
2.4	50	12	10	Tomato
2.4	50	12	10	Corn
2.4	47	12	9.4	Oat
2.4	50	6.0	20	Wheat
4.8	50	12	20	Cabbage
4.8	50	12	20	Soybean
4.8	50	12	20	Tomato
4.8	50	12	20	Corn
4.8	47	12	19	Oat
6.0	47	6.0	47	Wheat
12	50	12	50	Cabbage
12	50	12	50	Soybean
12	50	12	50	Tomato
12	50	12	50	Corn
12	47	12	47	Oat
15.6	47	6.0	120	Wheat

Stock Conc. (mg a.i./mL)	Volume Stock Applied (mL)	Total Weight of Sand and Soil (kg)	Test Concentration (mg a.i./kg)	Test Species
31.2	50	12	130	Cabbage
31.2	50	12	130	Soybean
31.2	50	12	130	Tomato
31.2	50	12	130	Corn
31.2	47	12	120	Oat
38.4	47	6.0	300	Wheat
76.8	50	12	320	Cabbage
76.8	50	12	320	Soybean
76.8	50	12	320	Tomato
76.8	50	12	320	Corn
76.8	47	12	300	Oat
192	50	12	800	Cabbage
192	50	12	800	Soybean
192	50	12	800	Oat

2.8 Test Initiation

The following table presents species replication and the number of seeds exposed per replicate during the study:

Species	Number of Replicates/Treatment	Number of Seeds/Replicate	Number of Seeds/Treatment
Cabbage	10	4	40
Corn	10	2	20
Oats	10	8	80
Soybean	10	2	20
Tomato	10	2	20
Wheat	5	8	40

The number of seeds selected per treatment was based on seed and expected plant size.

Approximately 1.2 kg of treated, solvent control or control soil was added to each pot.

All pots were labeled to identify the plant species, nominal concentration, replicate and study number. Control pots contained untreated sterile, sandy-loam. The soil in each pot was leveled and the appropriate number of seeds were impartially selected and planted at a depth of approximately 1 to 2 cm in each pot (20, 40 or 80 seeds per treatment and controls, depending upon species). The seeds were placed in a circular pattern around the inside perimeter of the pot.

As seedlings emerged, the plant located at the pot label constituted plant number one. The remaining plant positions were determined sequentially in a clockwise order, as they emerged. Approximately 100 mL of well water was added to each saucer. Thereafter, well water was added twice weekly (see Protocol Deviation). Control replicates were planted first, then solvent control, followed by the treatment levels (low to high concentration). Pots were grouped by species and placed in a random block format based on computer-generated random numbers.

2.9 Test Monitoring

Air temperature was controlled using a thermostatically-regulated heating/cooling system and was constantly monitored using a VWR minimum/maximum thermometer. Light intensity was measured daily using a Traceable radiometer/photometer held at an average maximum leaf height for each species. Light intensity was measured in footcandles and converted to lux, based on 1 footcandle = approximately 10.76 lux. Humidity was maintained through evaporation of water from the irrigation solution, and was monitored using a VWR Thermo-hygrometer.

Each control pot was observed daily until $\geq 50\%$ emergence was observed in the control. Seven, 14 and 21 days after 50% emergence in the control, the number of emerged plants, morphological abnormalities (e.g., chlorosis or necrosis of leaves) and mortalities were recorded. All control and treatment levels were terminated 21 days after $\geq 50\%$ emergence was determined in the controls. At test termination, the above-ground portion of the live plants within a pot were removed, placed in pre-weighed aluminum pans and dried in radiant heat ovens at $70 \pm 5^\circ\text{C}$ for at least three days before determining dry shoot weights to the nearest 0.0001 g.

2.10 Analytical Measurements

Prior to test initiation, a 3-mL sample of each dosing stock solution was removed by pipet for analysis of the bisphenol A concentration. A portion of each sample was diluted with acetonitrile to within the range of analytical standards used (e.g., 10 to 100 mg a.i./L).

Three quality control (QC) samples were also prepared at nominal concentrations which approximated the dosing stock solution concentration range and remained with the test samples throughout the analytical process. Analysis of the QC samples was used to judge the precision and quality control maintained during the analytical process.

All dosing stock solutions and QC samples were analyzed for bisphenol A using high performance liquid chromatographic system with ultraviolet detection (HPLC/UV) according to the following instrumental conditions:

Instrument:	Hewlett Packard quaternary solvent pump Series 1100 equipped with a Agilent 1200 Series degasser and autosampler, a Hewlett Packard variable wavelength detector, and Hewlett Packard ChemStation Version A.06.03 for data acquisition		
Column:	Agilent SB-C18, 3.5 μ m, 75 mm x 4.6 mm,		
Mobile Phase (A):	0.1% phosphoric acid in reagent water		
Mobile Phase (B):	100% acetonitrile		
Gradient:	<u>Time (min.)</u>	<u>Solvent A</u>	<u>Solvent B</u>
	0.00	95.0	5.0
	2.00	95.0	5.0
	12.0	0.00	100.0
	14.0	0.00	100.0
	15.0	95.00	5.0
Flow Rate:	1.4 mL/minute		
Injection Volume:	10 μ L		
Wavelength:	230 nm		
Column Temperature:	ambient		
Run Time:	15 minutes		
Equilibration Delay:	3.00 minutes		
Retention Time:	approximately 8.3 minutes		

2.11 Statistical Analysis

A t-Test (Sokal and Rohlf, 1981) was conducted to statistically compare the final percent emergence or dry shoot weight of the control to the solvent control data. If no significant difference was determined, control and solvent control data were pooled for comparison to treatment data. If a difference was determined between the control and solvent control data, the

solvent control was used for comparison with the treatment data. Percent inhibition of the treatment data was calculated relative to the appropriate control data.

Based on the results of statistical analyses performed for percent emergence and dry shoot weight, the No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) were determined. The data were first checked for normality using Chi-Square Test (Weber et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). If the data sets passed the tests for homogeneity and normality, then either Dunnett's Test (Dunnett, 1955, 1964) or Bonferroni's Test (Weber et al., 1989) was used to determine the NOEC. If the data did not pass the tests for homogeneity and normality, then Kruskal-Wallis' Test (Sokal and Rohlf, 1981) was used to determine the NOEC. All statistical determinations were made at the 95% level of certainty, except in the case of Chi-Square Test and Bartlett's Tests, where the 99% level of certainty was applied.

The EC25 and EC50 values (concentrations of test substance which reduced percent emergence and dry shoot weight by 25 and 50%, respectively) and the 95% confidence limits were determined using the IC_p method (Norberg-King, 1993). TOXSTAT[®] version 3.5 (Gulley et al., 1996) software was used to determine the NOEC and LOEC and calculate the EC values and 95% confidence limits. If less than the required response was observed (i.e., <25 or 50% response), the EC value was empirically estimated to be greater than the highest concentration tested.

3.0 RESULTS AND DISCUSSION

3.1 Test Monitoring

A summary of the environmental conditions monitored in the greenhouse during the definitive tests is presented in Table 2. The relative humidity ranged from 18 to 100% and temperature ranged from 24 to 42 °C. The OECD Guideline #208 recommends relative humidity and temperature ranges of 45 to 95% and 12 to 32 °C for greenhouse testing. Although the actual

conditions exceeded the recommended ranges, the conditions maintained have been used in past studies and no negative impact on the plants has been observed.

3.2 Analytical Results

The analytical results are presented for the dosing stock solution analyses in Table 3. Dosing stock measurements ranged from 85 to 100% of nominal concentration with the exception of one solution at 140% of nominal concentration. Since the measured stock solution concentrations closely approximated the desired nominal concentrations and the previous study (Springborn Smithers study number 13761.6123) indicated the soil mixing technique provided homogeneous dispersion of test substance in the sandy-loam, the results of this study are reported based on nominal application rate.

Analysis of the quality control samples resulted in measured concentrations which were consistent with the nominal fortified concentrations (0.0250, 0.0500 and 0.0750 mg a.i./mL) and ranged from 99.7 to 101% (N = 3) of nominal concentrations. Based on these results, it was established that the appropriate precision and quality control was maintained during the analyses of the stock solutions.

3.3 Biological Effects

The morphological abnormalities (e.g., chlorosis or necrosis of leaves) and mortality observed during the study, the percent emergence and dry shoot weights determined at test termination and the EC25, EC50, LOEC and NOEC values calculated for each species are presented in Table 4 through Table 28. The effects of bisphenol A on each species are discussed in the following sections. To minimize the redundancy, where pooled control data is mentioned, the control and solvent control data were determined to be statistically similar based on a t-Test ($p \leq 0.05$). If the control and solvent control data were statistically different, the treatment data were compared to the solvent control data.

Additionally, please note that negative inhibition throughout the text represents increased growth relative to the appropriate control data.

Cabbage – The test levels for cabbage were: 20, 50, 130, 320 and 800 mg a.i./kg. The morphological abnormalities and mortality observed at test termination are presented in Table 4. No dead plants or plants with morphological abnormalities were observed among plants in the control, solvent control, or the treatments tested. Only one seed exposed to the 320 mg a.i./kg treatment and no seeds exposed to the 800 mg a.i./kg treatment emerged by test termination. Several seeds exposed to the controls and the remaining treatment levels had not emerged by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 5 and Table 6, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 53 and 65%, respectively (pooled control = 59%). The pooled control percent emergence (59%) is slightly less than 70% germination requested in the OECD Guideline #208. The cabbage seed was new and packed for the 2007 growing season. Although the pooled control percent emergence was less than requested, the concentration-response curve was well defined and characterized the sensitivity of cabbage to bisphenol A. The day 21 mean percent seedling emergence for the 20, 50, 130, 320 and 800 mg a.i./kg treatments was 70, 88, 50, 2.5 and 0.0%, respectively, and yielded -19, -49, 15, 96 and 100% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control was 0.5574 and 0.2964 g, respectively. The day 21 shoot dry weight for the 20, 50, 130 and 320 mg a.i./kg treatments was 0.2165, 0.3058, 0.1622 and 0.0251 g, respectively, and yielded 27, -3, 45 and 92% inhibition, respectively, relative to the solvent control data.

The 21-day EC25, EC50, LOEC and NOEC values for percent emergence and dry shoot weight are presented in Table 7.

Summary - Seedling Emergence and Growth Test Endpoints with Cabbage

Biological Parameter	Based on Nominal Concentrations (mg a.i./kg)			
	EC25	EC50	LOEC	NOEC
21-Day Percent Emergence	130	190	320	130
21-Day Dry Shoot Weight	82	>130	130	50

Corn – The test levels for corn were: 3.8, 10, 20, 50, 130 and 320 mg a.i./kg. The morphological abnormalities and mortality observed at test termination are presented in Table 8. No dead plants or plants with morphological abnormalities were observed among plants in the control, solvent control, or the treatments tested. One seed exposed to the 10 mg a.i./kg treatment level, two seeds exposed to the 3.8 and 50 mg a.i./kg treatment levels and several seeds exposed to the controls and the remaining treatment levels did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 9 and Table 10, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 85 and 75%, respectively (pooled control = 80%). The day 21 mean percent seedling emergence for the 3.8, 10, 20, 50, 130 and 320 mg a.i./kg treatments was 90, 95, 80, 90, 65 and 80%, respectively, and yielded -13, -19, 0, -13, 19 and 0% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control was 1.4665 and 2.2619 g, respectively. The control and solvent control data were significantly different, therefore, the treatment data were compared to the solvent control data. The day 21 shoot dry weight for the 3.8, 10, 20, 50, 130 and 320 mg a.i./kg treatments was 1.6899, 1.7635, 1.9959, 1.8348, 1.1461 and 0.7472 g, respectively, and yielded 25, 22, 12, 19, 49 and 67% inhibition, respectively, relative to the solvent control data. Kruskal-Wallis' Test determined there was a significant reduction in shoot dry weight in the highest treatment tested, 320 mg a.i./kg, only. The inhibition noted in the remaining lower treatments is therefore not considered to be treatment-related. Mean shoot dry weight data for the four lowest treatments were actually greater than the mean control data.

The 21-day EC25, EC50, LOEC and NOEC values for percent emergence and dry shoot weight are presented in Table 11.

Summary - Seedling Emergence and Growth Test Endpoints with Corn

Biological Parameter	Based on Nominal Concentrations (mg a.i./kg)			
	EC25	EC50	LOEC	NOEC
21-Day Percent Emergence	>320	>320	>320	320
21-Day Dry Shoot Weight	83	160	320	130

Oat – The test levels for oat were: 9.4, 19, 47, 120, 300 and 800 mg a.i./kg. The morphological abnormalities and mortality observed at test termination are presented in Table 12. One dead plant was observed in the 800 mg a.i./kg treatment. Two seeds exposed to the 19 mg a.i./kg treatment level and several seeds exposed to the controls and the remaining treatment levels did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 13 and Table 14, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 64 and 88%, respectively (pooled control = 76%). The day 21 mean percent seedling emergence for the 9.4, 19, 47, 120, 300 and 800 mg a.i./kg treatments was 86, 98, 68, 18, 84 and 64%, respectively, and yielded -14, -29, 11, 77, -11 and 16% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control was 0.2776 and 0.2991 g, respectively (pooled control = 0.2889 g). The day 21 shoot dry weight for the 9.4, 19, 47, 120, 300 and 800 mg a.i./kg treatments was 0.2802, 0.3133, 0.2736, 0.0894, 0.0530 and 0.0100 g, respectively, and yielded 3, -8, 5, 69, 82 and 97% inhibition, respectively, relative to the pooled control data.

The 21-day EC25, EC50, LOEC and NOEC values for percent emergence and dry shoot weight are presented in Table 15.

Summary - Seedling Emergence and Growth Test Endpoints with Oat

Biological Parameter	Based on Nominal Concentrations (mg a.i./kg)			
	EC25	EC50	LOEC	NOEC
21-Day Percent Emergence	>800	>800	>800	800
21-Day Dry Shoot Weight	69	100	120	47

Soybean – The test levels for soybean were: 20, 50, 130, 320 and 800 mg a.i./kg. The morphological abnormalities and mortality observed at test termination are presented in Table 16. No dead plants or plants with morphological abnormalities were observed among plants in the control, solvent control, or the treatments tested. Several seeds exposed to the control and the 800 mg a.i./kg treatment level and two seeds exposed to the 130 and 320 mg a.i./kg treatment levels did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 17 and Table 18, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 75 and 100%, respectively. The day 21 mean percent seedling emergence for the 20, 50, 130, 320 and 800 mg a.i./kg treatments was 100, 100, 90, 90 and 75%, respectively, and yielded 0, 0, 10, 10 and 25% inhibition, respectively, relative to the solvent control data. The day 21 mean shoot dry weight for the control and solvent control was 2.3052 and 1.9170 g, respectively (pooled control = 2.1009 g). The day 21 shoot dry weight for the 20, 50, 130, 320 and 800 mg a.i./kg treatments was 1.4529, 2.0287, 1.7787, 1.4084 and 0.1869 g, respectively, and yielded 31, 3, 15, 33 and 91% inhibition, respectively, relative to the pooled control data.

The 21-day EC25, EC50, LOEC and NOEC values for percent emergence and dry shoot weight are presented in Table 19.

Summary - Seedling Emergence and Growth Test Endpoints with Soybean

Biological Parameter	Based on Nominal Concentrations (mg a.i./kg)			
	EC25	EC50	LOEC	NOEC
21-Day Percent Emergence	650	>800	>800	800
21-Day Dry Shoot Weight	220	460	800	320

Tomato – The test levels for tomato were: 4.0, 10, 20, 50, 130 and 320 mg a.i./kg. The morphological abnormalities and mortality observed at test termination are presented in Table 20. No dead plants or plants with morphological abnormalities were observed among plants in the control, solvent control, or the treatments tested. One seed exposed to the 20 mg a.i./kg treatment level, two seeds exposed to the control and the 50 and 130 mg a.i./kg treatment levels and several seeds exposed to the solvent control and the remaining treatment levels did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 21 and Table 22, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 90 and 80%, respectively (pooled control = 85%). The day 21 mean percent seedling emergence for the 4.0, 10, 20, 50, 130 and 320 mg a.i./kg treatments was 85, 85, 95, 90, 90 and 25%, respectively, and yielded 0, 0, -12, -6, -6 and 71% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control was 1.1461 and 1.1154 g, respectively (pooled control = 1.1308 g). The day 21 shoot dry weight for the 4.0, 10, 20, 50, 130 and 320 mg a.i./kg treatments was 1.0774, 0.9528, 0.8137, 0.6790, 0.1568 and 0.0206 g, respectively, and yielded 5, 16, 28, 40, 86 and 98% inhibition, respectively, relative to the pooled control data.

The 21-day EC25, EC50, LOEC and NOEC values for percent emergence and dry shoot weight are presented in Table 23.

Summary - Seedling Emergence and Growth Test Endpoints with Tomato

Biological Parameter	Based on Nominal Concentrations (mg a.i./kg)			
	EC25	EC50	LOEC	NOEC
21-Day Percent Emergence	190	260	320	130
21-Day Dry Shoot Weight	19	67	50	20

Wheat – The test levels for wheat were: 3.8, 9.4, 20, 47, 120 and 300 mg a.i./kg. The morphological abnormalities and mortality observed at test termination are presented in Table

24. No dead plants or plants with morphological abnormalities were observed among plants in the control, solvent control, or the treatments tested. One seed exposed to the solvent control and the 3.8, 20, 120 and 300 mg a.i./kg treatment levels did not emerge by test termination.

The mean percent emergence and dry shoot weight data determined at test termination for each test concentration and the controls are presented in Table 25 and Table 26, respectively. The day 21 mean percent seedling emergence for the control and solvent control was 100 and 98%, respectively (pooled control = 99%). The day 21 mean percent seedling emergence for the 3.8, 9.4, 20, 47, 120 and 300 mg a.i./kg treatments was 98, 100, 98, 100, 98 and 98%, respectively, and yielded 1, -1, 1, -1, 1 and 1% inhibition, respectively, relative to the pooled control data. The day 21 mean shoot dry weight for the control and solvent control was 0.1900 and 0.1609 g, respectively. The control and solvent control data were significantly different, therefore, the treatment data were compared to the solvent control data. The day 21 shoot dry weight for the 3.8, 9.4, 20, 47, 120 and 300 mg a.i./kg treatments was 0.1685, 0.1541, 0.1858, 0.1678, 0.1230 and 0.0297 g, respectively, and yielded -5, 4, -15, -4, 24 and 82% inhibition, respectively, relative to the solvent control data.

The 21-day EC25, EC50, LOEC and NOEC values for percent emergence and dry shoot weight are presented in Table 27.

Summary - Seedling Emergence and Growth Test Endpoints with Wheat

Biological Parameter	Based on Nominal Concentrations (mg a.i./kg)			
	EC25	EC50	LOEC	NOEC
21-Day Percent Emergence	>300	>300	>300	300
21-Day Dry Shoot Weight	120	200	120	47

4.0 CONCLUSIONS

The results demonstrated that in general dry shoot weight was a more sensitive indicator of the effects of bisphenol A than percent emergence, with oat and tomato exhibiting the most sensitivity (EC50 values of 100 and 67 mg a.i./kg, respectively). The EC25, EC50, LOEC and NOEC values for percent seedling emergence and shoot dry weight are summarized in Table 28.

PROTOCOL DEVIATION

The protocol states that dilute water soluble fertilizer will be provided to each pot by subirrigation twice weekly during the test and on the remaining days the pots will receive well water. During this study, the fertilizer solution was inadvertently not applied to any pots on the last scheduled application and well water was substituted that day. Since all plants received an equivalent amount of nutrients and water throughout the study, this deviation does not impact the results or interpretation of the study.

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Table 1. Historical data for seeds used in the definitive seedling emergence and growth tests with bisphenol A.

Species ^a	Variety ^a	Date Packed ^a	Date Received	Supplier Lot No. ^a	SSL Lot No.	% Germ. ^a	Date Tested ^a
Cabbage	Cairo ^b	NA ^f	2/23/07	NA	22307	NA	NA
Corn	Truckers Favorite ^c	NA	10/5/06	NA	100506	90	11/05
Oat	Jerry ^d	2006	10/10/06	05TI	101006C	98	12/05
Soybean	Edible Early Hakucho ^b	NA	4/6/07	QV09	040607	96	1/07
Tomato	Celebrity Hybrid ^b	NA	2/28/07	NA	022807	NA	NA
Wheat	VNS ^e	NA	12/18/06	TRAR-35927	121806	96	8/1/06

^a Information provided by the supplier.^b Supplied by Park Seed Company, Greenwood, South Carolina.^c Supplied by Carolina Biological Supply Company, Burlington, North Carolina.^d Supplied by Seeds of Change, Santa Fe, New Mexico.^e Supplied by Granite Seed Company, Lehi, Utah.^f NA = Not Available.

All seeds refrigerated at approximately 4 °C in the dark until test initiation.

Table 2. Environmental conditions measured in the greenhouse during the seedling emergence and growth tests with bisphenol A.

Parameter	Range ^a
Relative Humidity (%)	18 – 100
Temperature (°C)	24 – 42
Light Intensity ^b (ftc)	590 – 8200
Light Intensity ^b (lux)	6300 – 88000

^a Rounded to two significant figures.

^b For light intensity, overall range was based on daily readings taken in multiple locations in the greenhouse.

Table 3. Concentrations of bisphenol A measured in the dosing stock solutions prior to the seedling emergence test.

Dosing Stock Solutions (mg a.i./mL)	Measured Concentration (mg a.i./mL)	
	Day -2	Percent of Nominal
Solvent Control	< 0.0298 ^a	NA ^b
0.48	0.407	84.7
0.96	0.816	84.9
1.2	1.09	90.9
2.4	3.32	138
4.8	4.82	100
6.0	5.80	96.7
12	11.5	96.0
16	15.9	102
31	30.7	98.4
38	39.3	102
77	75.0	97.6
192	195	102
QC ^c #1 (0.0250)	0.0249	99.7
QC #2 (0.0500)	0.0502	100
QC #3 (0.0750)	0.0754	101

^a Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each sample is dependent upon the sample volume, dilution factor and standard concentration range.

^b NA = Not Applicable.

^c QC = Quality Control sample.

NOTE: Prior to analysis, stock solutions were diluted to a similar concentration of the QC samples.

Table 4. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for cabbage (*Brassica oleracea*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Number	Plant Condition at Test Termination									
		1	2	3	4	5	6	7	8	9	10
Control	1	H	H	H	H	H	H	H	Ne	H	H
	2	H	Ne	Ne	H	H	Ne	H	Ne	H	H
	3	Ne	Ne	Ne	H	H	Ne	Ne	Ne	H	H
	4	Ne	Ne	Ne	Ne	H	Ne	Ne	Ne	H	Ne
Solvent Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	Ne	H	H	H	H
	3	H	Ne	Ne	H	H	Ne	Ne	H	Ne	Ne
	4	H	Ne	Ne	Ne	H	Ne	Ne	H	Ne	Ne
20	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	Ne	H	H	H	H	H	H
	3	H	H	H	Ne	H	H	H	Ne	H	Ne
	4	Ne	Ne	H	Ne	Ne	H	Ne	Ne	Ne	Ne
50	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	Ne	H	H	H	H	Ne
	4	H	H	H	Ne	Ne	H	H	H	H	Ne
130	1	H	H	H	H	H	Ne	Ne	H	H	H
	2	H	Ne	H	H	H	Ne	Ne	H	H	H
	3	H	Ne	Ne	H	H	Ne	Ne	Ne	H	Ne
	4	Ne	Ne	Ne	Ne	H	Ne	Ne	Ne	Ne	Ne
320	1	H	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	2	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	3	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	4	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
800	1	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	2	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	3	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	4	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 5. Percent emergence of cabbage (*Brassica oleracea*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	2	50	53	NA ^b
	2	3	25		
	3	3	25		
	4	1	75		
	5	0	100		
	6	3	25		
	7	2	50		
	8	4	0		
	9	0	100		
	10	1	75		
Solvent Control	1	0	100	65	NA
	2	2	50		
	3	2	50		
	4	1	75		
	5	0	100		
	6	3	25		
	7	2	50		
	8	0	100		
	9	2	50		
	10	2	50		
Pooled Control				59	NA
20	1	1	75	70	-19
	2	1	75		
	3	0	100		
	4	3	25		
	5	1	75		
	6	0	100		
	7	1	75		
	8	2	50		
	9	1	75		
	10	2	50		
50	1	0	100	88	-49
	2	0	100		
	3	0	100		
	4	1	75		
	5	2	50		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	2	50		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.^c Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 5. (continued) Percent emergence of cabbage (*Brassica oleracea*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
130	1	1	75	50	15
	2	3	25		
	3	2	50		
	4	1	75		
	5	0	100		
	6	4	0		
	7	4	0		
	8	2	50		
	9	1	75		
	10	2	50		
320	1	3	25	2.5 ^c	96
	2	4	0		
	3	4	0		
	4	4	0		
	5	4	0		
	6	4	0		
	7	4	0		
	8	4	0		
	9	4	0		
	10	4	0		
800	1	4	0	0.0 ^c	100
	2	4	0		
	3	4	0		
	4	4	0		
	5	4	0		
	6	4	0		
	7	4	0		
	8	4	0		
	9	4	0		
	10	4	0		

^a Percent inhibition relative to the pooled control.

^b NA = Not Applicable.

^c Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 6. Shoot dry weight of cabbage (*Brassica oleracea*) exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	0.4180	0.1651	0.5574	0.3404	NA ^d
	2	1.0901	NA			
	3	0.9622	NA			
	4	0.5196	0.1964			
	5	0.2961	0.0600			
	6	0.7923	NA			
	7	0.6005	NA			
	8	NA	NA			
	9	0.2954	0.1665			
	10	0.0430	0.0308			
Solvent Control	1	0.2522	0.0266	0.2964	0.0882	NA
	2	0.3494	0.0980			
	3	0.2999	0.0153			
	4	0.2743	0.0490			
	5	0.2139	0.1097			
	6	0.3616	NA			
	7	0.2299	0.0456			
	8	0.2046	0.1126			
	9	0.4980	0.1188			
	10	0.2804	0.1460			
20	1	0.0902	0.1336	0.2165 ^{ef}	0.0915	27
	2	0.1957	0.1311			
	3	0.1845	0.1376			
	4	0.4236	NA			
	5	0.1747	0.1869			
	6	0.1786	0.1372			
	7	0.2188	0.1550			
	8	0.2860	0.0784			
	9	0.1479	0.1751			
	10	0.2655	0.2237			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the solvent control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the solvent control, based on Bonferroni's Test.^f Inhibition at 20 mg a.i./kg not considered treatment-related since the next highest concentration is not significantly reduced compared to the solvent control.^g Shoot dry weight at 320 and 800 mg a.i./kg treatment levels was not statistically analyzed due to a lack of emerged plants.

Table 6. (continued) Shoot dry weight of cabbage (*Brassica oleracea*) exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
50	1	0.2665	0.1288	0.3058	0.0457	-3
	2	0.3590	0.1889			
	3	0.2595	0.1087			
	4	0.3077	0.0762			
	5	0.3591	0.0690			
	6	0.2887	0.1562			
	7	0.3580	0.1710			
	8	0.2918	0.1972			
	9	0.2328	0.1611			
	10	0.3353	0.1061			
130	1	0.1692	0.0533	0.1622 ^e	0.0570	45
	2	0.2318	NA			
	3	0.1396	0.0084			
	4	0.1691	0.0602			
	5	0.1710	0.1052			
	6	NA	NA			
	7	NA	NA			
	8	0.0373	NA			
	9	0.1942	0.0246			
	10	0.1859	0.0630			
320	1	0.0251	NA	0.0251 ^g	NA	92
	2	NA	NA			
	3	NA	NA			
	4	NA	NA			
	5	NA	NA			
	6	NA	NA			
	7	NA	NA			
	8	NA	NA			
	9	NA	NA			
	10	NA	NA			
800	1	NA	NA	NA	NA	NA
	2	NA	NA			
	3	NA	NA			
	4	NA	NA			
	5	NA	NA			
	6	NA	NA			
	7	NA	NA			
	8	NA	NA			
	9	NA	NA			
	10	NA	NA			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the solvent control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the solvent control, based on Bonferroni's Test.^f Inhibition at 20 mg a.i./kg not considered treatment-related since the next highest concentration was not significantly reduced compared to the solvent control.^g Shoot dry weight at 320 and 800 mg a.i./kg treatment levels was not statistically analyzed due to a lack of emerged plants.

Table 7. **The EC25, EC50, NOEC and LOEC values for bisphenol A calculated from the 21-day toxicity test results (percent emergence and dry shoot weight) with cabbage (*Brassica oleracea*).**

Percent Emergence	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	130	190	320	130
95% confidence limits:	83 - 180	120 - 230		
Dry Shoot Weight	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	82	>130	130	50
95% confidence limits:	52 - 120	NA ^a		

^a NA = Not Applicable. EC50 value was empirically estimated, therefore, 95% confidence limits could not be calculated.

Table 8. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for corn (*Zea mays*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Condition at Test Termination										
	Plant Number	1	2	3	4	Replicate					
Control	1	H	H	H	H	H	H	H	H	H	H
	2	Ne	H	Ne	H	Ne	H	H	H	H	H
Solvent Control	1	Ne	H	H	H	H	H	H	H	H	H
	2	Ne	Ne	H	Ne	H	H	H	H	Ne	H
3.8	1	H	H	H	H	H	H	H	H	H	H
	2	Ne	H	H	Ne	H	H	H	H	H	H
10	1	H	H	H	H	H	H	H	H	H	H
	2	Ne	H	H	H	H	H	H	H	H	H
20	1	Ne	H	H	H	H	H	H	H	H	H
	2	Ne	H	H	H	H	H	Ne	H	Ne	H
50	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	Ne	H	H	Ne	H	H	H	H
130	1	Ne	H	H	H	H	H	H	Ne	H	H
	2	Ne	Ne	H	H	H	H	Ne	Ne	Ne	H
320	1	H	Ne	H	H	H	H	H	H	H	Ne
	2	H	Ne	H	H	H	H	H	H	H	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 9. Percent emergence of corn (*Zea mays*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	1	50	85	NA ^b
	2	0	100		
	3	1	50		
	4	0	100		
	5	1	50		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
Solvent Control	1	2	0	75	NA
	2	1	50		
	3	0	100		
	4	1	50		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	1	50		
	10	0	100		
Pooled Control				80	NA
3.8	1	1	50	90	-13
	2	0	100		
	3	0	100		
	4	1	50		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
10	1	1	50	95	-19
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.

Table 9. (continued) Percent emergence of corn (*Zea mays*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
20	1	2	0	80	0
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	1	50		
	8	0	100		
	9	1	50		
	10	0	100		
50	1	0	100	90	-13
	2	0	100		
	3	1	50		
	4	0	100		
	5	0	100		
	6	1	50		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
130	1	2	0	65	19
	2	1	50		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	1	50		
	8	2	0		
	9	1	50		
	10	0	100		
320	1	0	100	80	0
	2	2	0		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	2	0		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.

Table 10. Shoot dry weight of corn (*Zea mays*) exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					Inhibition (%) ^c
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	
Control	1	1.3029	NA	1.4665	0.5348	NA ^d
	2	1.3005	0.7152			
	3	1.8827	NA			
	4	1.5455	1.4649			
	5	2.5480	NA			
	6	0.4669	0.0933			
	7	1.4215	1.3318			
	8	1.3708	0.0963			
	9	1.1347	1.0921			
	10	1.6916	0.2178			
Solvent Control	1	NA	NA	2.2619	1.0197	NA
	2	3.2558	NA			
	3	1.4345	0.4173			
	4	3.3880	NA			
	5	1.8502	1.4786			
	6	1.5026	0.0117			
	7	1.6124	0.1710			
	8	1.3444	0.5107			
	9	4.0623	NA			
	10	1.9072	0.8592			
3.8	1	2.8251	NA	1.6899	0.4695	25
	2	1.5142	0.0493			
	3	1.7308	0.9994			
	4	1.4424	NA			
	5	1.8243	1.6186			
	6	1.7248	0.3307			
	7	1.7741	0.4030			
	8	0.9869	1.2229			
	9	1.6699	1.3518			
	10	1.4067	0.8738			
10	1	1.6429	NA	1.7635	0.1831	22
	2	2.1594	0.4223			
	3	1.6162	0.3727			
	4	1.6386	0.6887			
	5	2.0063	0.6867			
	6	1.8286	1.5806			
	7	1.7193	1.2781			
	8	1.6980	0.5660			
	9	1.7053	0.7584			
	10	1.6206	1.0736			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the solvent control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the solvent control, based on Kruskal-Wallis' Test.

Table 10. (continued) Shoot dry weight of corn (*Zea mays*) exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					Inhibition (%) ^c
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	
20	1	NA	NA	1.9959	0.7395	12
	2	1.8306	0.7555			
	3	1.2589	0.0499			
	4	1.8370	0.4032			
	5	1.7864	0.1930			
	6	1.5905	1.0541			
	7	3.1411	NA			
	8	1.4930	0.5118			
	9	3.3708	NA			
	10	1.6550	0.4564			
50	1	1.5629	0.0110	1.8348	0.4213	19
	2	1.1781	0.4592			
	3	2.1944	NA			
	4	1.5823	0.3226			
	5	2.1145	0.5271			
	6	2.5775	NA			
	7	1.4060	0.4603			
	8	1.7028	0.1390			
	9	2.0540	0.4429			
	10	1.9756	0.3083			
130	1	NA	NA	1.1461	1.0883	49
	2	0.0331	NA			
	3	0.9985	0.4643			
	4	1.2916	0.1834			
	5	0.7283	0.3432			
	6	1.0867	0.2311			
	7	3.4623	NA			
	8	NA	NA			
	9	0.0082	NA			
	10	1.5605	0.4692			
320	1	0.6915	0.3504	0.7472 ^e	0.1563	67
	2	NA	NA			
	3	0.7525	0.1890			
	4	0.5014	0.0800			
	5	0.8562	0.2314			
	6	0.7794	0.0735			
	7	0.9967	0.0606			
	8	0.5817	0.0193			
	9	0.8180	0.2442			
	10	NA	NA			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the solvent control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the solvent control, based on Kruskal-Wallis' Test.

Table 11. **The EC25, EC50, NOEC and LOEC values for bisphenol A calculated from the 21-day toxicity test results (percent emergence and dry shoot weight) with corn (*Zea mays*).**

Percent Emergence	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	>320	>320	>320	320
95% confidence limits:	NA ^a	NA ^a		

Dry Shoot Weight	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	83	160	320	130
95% confidence limits:	14 – 180	80 - 280		

^a NA = Not Applicable. EC25 and EC50 values were empirically estimated, therefore, 95% confidence limits could not be calculated.

Table 12. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Number	Plant Condition at Test Termination									
		1	2	3	4	Replicate		7	8	9	10
Control	1	H	H	H	H	H	H	H	Ne	H	H
	2	H	H	H	H	H	H	H	Ne	H	H
	3	H	H	H	H	H	H	H	Ne	Ne	H
	4	H	H	H	H	H	H	H	Ne	Ne	H
	5	Ne	H	H	H	H	H	Ne	Ne	Ne	H
	6	Ne	Ne	H	H	H	H	Ne	Ne	Ne	H
	7	Ne	Ne	H	Ne	H	H	Ne	Ne	Ne	Ne
	8	Ne	Ne	H	Ne	H	H	Ne	Ne	Ne	Ne
Solvent Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	Ne	H	H	H	H	H	H	H	H
	3	H	Ne	H	H	H	H	H	H	H	H
	4	H	Ne	H	H	H	H	H	H	H	H
	5	H	Ne	H	H	H	H	H	H	H	H
	6	Ne	Ne	H	H	H	H	H	H	H	H
	7	Ne	Ne	H	H	H	H	H	H	H	H
	8	Ne	Ne	H	H	H	H	H	H	H	H
9.4	1	H	H	H	H	H	Ne	H	H	H	H
	2	H	H	H	H	H	Ne	H	H	H	H
	3	H	H	H	H	H	Ne	H	H	H	H
	4	H	H	H	H	H	Ne	H	H	H	H
	5	H	H	H	H	H	Ne	H	H	H	H
	6	H	H	H	H	H	Ne	H	H	H	H
	7	H	H	H	H	H	Ne	H	H	H	H
	8	H	Ne	Ne	H	H	Ne	H	Ne	H	H
19	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	H	H	H	H	H	H
	4	H	H	H	H	H	H	H	H	H	H
	5	H	H	H	H	H	H	H	H	H	H
	6	H	H	H	H	H	H	H	H	H	H
	7	H	H	H	H	H	H	H	H	H	H
	8	H	H	H	Ne	H	Ne	H	H	H	H

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 12. (continued) Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Number	Plant Condition at Test Termination									
		1	2	3	4	Replicate		7	8	9	10
47	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	H	H	H	H	H	H
	4	H	H	H	H	H	H	H	H	H	Ne
	5	H	Ne	Ne	H	H	H	H	H	H	Ne
	6	Ne	Ne	Ne	Ne	H	H	H	Ne	H	Ne
	7	Ne	Ne	Ne	Ne	H	Ne	H	Ne	H	Ne
	8	Ne	Ne	Ne	Ne	Ne	Ne	H	Ne	Ne	Ne
120	1	Ne	Ne	Ne	Ne	H	H	H	H	H	H
	2	Ne	Ne	Ne	Ne	H	Ne	Ne	H	Ne	H
	3	Ne	Ne	Ne	Ne	H	Ne	Ne	H	Ne	H
	4	Ne	Ne	Ne	Ne	H	Ne	Ne	H	Ne	Ne
	5	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	6	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	7	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
	8	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne
300	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	H	H	H	H	H	Ne
	4	H	H	H	H	H	H	H	H	H	Ne
	5	H	H	H	H	H	H	H	Ne	H	Ne
	6	H	H	H	H	H	H	H	Ne	H	Ne
	7	H	Ne	H	H	H	H	H	Ne	H	Ne
	8	Ne	Ne	H	H	H	H	H	Ne	H	Ne
800	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
	3	H	H	H	H	H	H	Ne	H	Ne	H
	4	H	H	H	H	H	H	Ne	H	Ne	H
	5	H	H	Ne	H	H	H	Ne	H	Ne	H
	6	Ne	H	Ne	H	H	Dp	Ne	Ne	Ne	H
	7	Ne	H	Ne	H	Ne	Ne	Ne	Ne	Ne	H
	8	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 13. Percent emergence of oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	4	50	64	NA ^b
	2	3	63		
	3	0	100		
	4	2	75		
	5	0	100		
	6	0	100		
	7	4	50		
	8	8	0		
	9	6	25		
	10	2	75		
Solvent Control	1	3	63	88	NA
	2	7	13		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
Pooled Control				76	NA
9.4	1	0	100	86	-14
	2	1	88		
	3	1	88		
	4	0	100		
	5	0	100		
	6	8	0		
	7	0	100		
	8	1	88		
	9	0	100		
	10	0	100		
19	1	0	100	98	-29
	2	0	100		
	3	0	100		
	4	1	88		
	5	0	100		
	6	1	88		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.^c Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test. However, since the inhibition at the 300 and 800 mg a.i./kg treatment levels was not significantly reduced compared to the pooled control, the effect at 120 mg a.i./kg is not considered treatment-related.

Table 13. (continued) Percent emergence of oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
47	1	3	63	68	11
	2	4	50		
	3	4	50		
	4	3	63		
	5	1	88		
	6	2	75		
	7	0	100		
	8	3	63		
	9	1	88		
	10	5	38		
120	1	8	0	18 ^c	77
	2	8	0		
	3	8	0		
	4	8	0		
	5	4	50		
	6	7	13		
	7	7	13		
	8	4	50		
	9	7	13		
	10	5	38		
300	1	1	88	84	-11
	2	2	75		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	4	50		
	9	0	100		
	10	6	25		
800	1	3	63	64	16
	2	1	88		
	3	4	50		
	4	1	88		
	5	2	75		
	6	2	75		
	7	6	25		
	8	3	63		
	9	6	25		
	10	1	88		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.^c Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test. However, since the inhibition at the 300 and 800 mg a.i./kg treatment levels was not significantly reduced compared to the pooled control, the effect at 120 mg a.i./kg is not considered treatment-related.

Table 14. Shoot dry weight of oat (*Avena sativa*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	0.1819	0.0548	0.2776	0.0568	NA ^d
	2	0.3745	0.0188			
	3	0.2770	0.0443			
	4	0.2292	0.1398			
	5	0.2736	0.0933			
	6	0.2618	0.0452			
	7	0.2606	0.1144			
	8	NA	NA			
	9	0.3003	0.0191			
	10	0.3397	0.0798			
Solvent Control	1	0.3684	0.1942	0.2991	0.0464	NA
	2	0.3088	NA			
	3	0.3035	0.0827			
	4	0.2281	0.1225			
	5	0.3081	0.0950			
	6	0.3220	0.0657			
	7	0.2149	0.0707			
	8	0.3032	0.0526			
	9	0.2941	0.0414			
	10	0.3404	0.0435			
Pooled Control 9.4	1	0.2996	0.1127	0.2889 0.2802	0.0513 0.0247	NA 3
	2	0.2563	0.1417			
	3	0.3237	0.0416			
	4	0.2823	0.0492			
	5	0.2796	0.0509			
	6	NA	NA			
	7	0.2884	0.0657			
	8	0.2369	0.0587			
	9	0.2719	0.0860			
	10	0.2829	0.0639			
19	1	0.2795	0.1199	0.3133	0.0269	-8
	2	0.3204	0.1390			
	3	0.3017	0.0458			
	4	0.3516	0.0643			
	5	0.3054	0.0907			
	6	0.2708	0.1090			
	7	0.3204	0.0718			
	8	0.3190	0.0456			
	9	0.3094	0.0857			
	10	0.3550	0.0486			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 14. (continued) Shoot dry weight of oat (*Avena sativa*) exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
47	1	0.3338	0.0681	0.2736	0.0532	5
	2	0.2852	0.0386			
	3	0.2594	0.1163			
	4	0.3571	0.0949			
	5	0.2394	0.0758			
	6	0.3044	0.0475			
	7	0.2846	0.1335			
	8	0.2250	0.1738			
	9	0.2737	0.1251			
	10	0.1738	0.1376			
120	1	NA	NA	0.0894 ^e	0.0775	69
	2	NA	NA			
	3	NA	NA			
	4	NA	NA			
	5	0.0425	0.0412			
	6	0.0149	NA			
	7	0.1133	NA			
	8	0.1610	0.0307			
	9	0.0122	NA			
	10	0.1924	0.0889			
300	1	0.0320	0.0097	0.0530 ^e	0.0230	82
	2	0.0439	0.0178			
	3	0.0438	0.0211			
	4	0.0788	0.0470			
	5	0.0596	0.0440			
	6	0.0935	0.0268			
	7	0.0489	0.0216			
	8	0.0298	0.0164			
	9	0.0747	0.0362			
	10	0.0249	0.0122			
800	1	0.0087	0.0023	0.0100 ^e	0.0026	97
	2	0.0130	0.0023			
	3	0.0059	0.0031			
	4	0.0114	0.0035			
	5	0.0121	0.0063			
	6	0.0136	0.0024			
	7	0.0068	0.0047			
	8	0.0106	0.0022			
	9	0.0087	0.0050			
	10	0.0093	0.0037			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 15. **The EC25, EC50, NOEC and LOEC values for bisphenol A calculated from the 21-day toxicity test results (percent emergence and dry shoot weight) with oat (*Avena sativa*).**

Percent Emergence	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	>800	>800	>800	800
95% confidence limits:	NA ^a	NA ^a		
Dry Shoot Weight	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	69	100	120	47
95% confidence limits:	57 - 81	87 - 130		

^a NA = Not Applicable. EC25 and EC50 values were empirically estimated, therefore, 95% confidence limits could not be calculated.

Table 16. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for soybean (*Glycine max*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Condition at Test Termination										
	Plant Number	1	2	3	4	Replicate		7	8	9	10
Control	1	Ne	H	H	H	H	H	H	H	H	H
	2	Ne	Ne	Ne	H	H	H	H	H	Ne	H
Solvent Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
20	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
50	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	H	H	H	H	H
130	1	H	H	H	H	H	H	H	H	H	H
	2	Ne	Ne	H	H	H	H	H	H	H	H
320	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	Ne	H	H	H	H	Ne	H
800	1	Ne	H	H	H	H	H	H	H	H	H
	2	Ne	Ne	H	H	H	H	H	H	Ne	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 17. Percent emergence of soybean (*Glycine max*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	2	0	75	NA ^b
	2	1	50		
	3	1	50		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	1	50		
	9	0	100		
	10	0	100		
Solvent Control	1	0	100	100	NA
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
20	1	0	100	100	0
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
50	1	0	100	100	0
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		

^a Percent inhibition relative to the solvent control.

^b NA = Not Applicable.

Table 17. (continued) Percent emergence of soybean (*Glycine max*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
130	1	1	50	90	10
	2	1	50		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
320	1	0	100	90	10
	2	0	100		
	3	0	100		
	4	1	50		
	5	0	100		
	6	0	100		
	7	0	100		
	8	1	50		
	9	0	100		
	10	0	100		
800	1	2	0	75	25
	2	1	50		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	1	50		
	9	1	50		
	10	0	100		

^a Percent inhibition relative to the solvent control.^b NA = Not Applicable.

Table 18. Shoot dry weight of soybean (*Glycine max*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	NA	NA	2.3052	0.8894	NA ^d
	2	3.9292	NA			
	3	3.4095	NA			
	4	1.4394	0.0995			
	5	1.9361	0.4249			
	6	2.1337	0.3874			
	7	2.0533	0.3369			
	8	2.6900	NA			
	9	1.9564	0.0398			
	10	1.1993	0.4818			
Solvent Control	1	1.7843	0.0257	1.9170	0.2157	NA
	2	2.0195	0.0675			
	3	1.7795	0.1018			
	4	1.5918	0.1138			
	5	2.1148	0.4572			
	6	2.0194	0.0146			
	7	1.5741	0.3985			
	8	2.1392	0.0585			
	9	2.0696	0.1315			
	10	2.0782	0.7475			
Pooled Control 20				2.1009	0.6438	
	1	1.3646	0.5882			
	2	2.1414	0.0675			
	3	2.5259	0.0209			
	4	1.0567	0.0085			
	5	1.4947	0.0183			
	6	1.0075	0.4119			
	7	1.1267	0.1283			
	8	1.1227	0.4341			
	9	2.4878	0.1029			
50	10	0.2016	0.1544	2.0287	0.4238	3
	1	2.4232	0.5998			
	2	2.0156	0.3756			
	3	2.0194	0.0344			
	4	1.7405	1.1436			
	5	2.2594	0.1588			
	6	2.0544	0.2890			
	7	1.2149	0.2176			
	8	2.4577	0.2821			
	9	1.5633	0.4127			
	10	2.5393	0.0798			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 18. (continued) Shoot dry weight of soybean (*Glycine max*) exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
130	1	2.7403	NA	1.7787	0.7694	15
	2	2.8888	0.0000			
	3	1.0179	0.0639			
	4	1.5477	0.6860			
	5	1.0061	1.0699			
	6	2.2863	0.0544			
	7	0.9400	0.2547			
	8	1.0309	0.3491			
	9	2.4136	0.1732			
	10	1.9158	0.8067			
320	1	1.5010	0.1310	1.4084	0.3400	33
	2	0.6181	0.7010			
	3	1.7673	0.2227			
	4	1.6180	NA			
	5	1.3756	0.2937			
	6	1.2472	0.5967			
	7	1.8151	0.3275			
	8	1.4048	NA			
	9	1.2296	0.1922			
	10	1.5075	0.0662			
800	1	NA	NA	0.1869 ^e	0.1250	91
	2	0.0681	NA			
	3	0.1297	0.0421			
	4	0.1113	0.0713			
	5	0.2274	0.0208			
	6	0.1334	0.0598			
	7	0.2822	0.0421			
	8	0.3823	NA			
	9	0.0160	NA			
	10	0.3315	0.0085			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the pooled control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 19. **The EC25, EC50, NOEC and LOEC values for bisphenol A calculated from the 21-day toxicity test results (percent emergence and dry shoot weight) with soybean (*Glycine max*).**

Percent Emergence	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	650	>800	> 800	800
95% confidence limits:	370 – 800	NA ^a		

Dry Shoot Weight	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	220	460	800	320
95% confidence limits:	72 – 360	370 - 520		

^a NA = Not Applicable. EC50 value was empirically estimated, therefore, 95% confidence limits could not be calculated.

Table 20. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Condition at Test Termination										
	Plant Number	1	2	3	4	Replicate		7	8	9	10
Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	Ne	H	H	H	Ne	H	H	H
Solvent Control	1	H	H	H	H	H	H	H	H	H	H
	2	H	Ne	H	H	H	Ne	H	H	Ne	Ne
4.0	1	H	H	H	H	H	H	H	H	H	H
	2	H	Ne	Ne	H	H	H	H	H	H	Ne
10	1	H	H	H	H	H	Ne	H	H	H	H
	2	H	H	H	Ne	H	Ne	H	H	H	H
20	1	H	H	H	H	H	H	H	H	H	H
	2	Ne	H	H	H	H	H	H	H	H	H
50	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	Ne	H	Ne	H	H	H
130	1	H	H	H	H	H	H	H	H	H	H
	2	H	H	H	H	H	Ne	H	H	H	Ne
320	1	H	H	Ne	Ne	H	Ne	Ne	H	H	Ne
	2	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne	Ne

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 21. Percent emergence of tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	0	100	90	NA ^b
	2	0	100		
	3	1	50		
	4	0	100		
	5	0	100		
	6	0	100		
	7	1	50		
	8	0	100		
	9	0	100		
	10	0	100		
Solvent Control	1	0	100	80	NA
	2	1	50		
	3	0	100		
	4	0	100		
	5	0	100		
	6	1	50		
	7	0	100		
	8	0	100		
	9	1	50		
	10	1	50		
Pooled Control				85	NA
4.0	1	0	100	85	0
	2	1	50		
	3	1	50		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	1	50		
10	1	0	100	85	0
	2	0	100		
	3	0	100		
	4	1	50		
	5	0	100		
	6	2	0		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		

^a Percent inhibition relative to the pooled control.

^b NA = Not Applicable.

^c Significantly reduced compared to the pooled control, based on Bonferroni's Test.

Table 21. (continued) Percent emergence of tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
20	1	1	50	95	-12
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	0	100		
	7	0	100		
	8	0	100		
	9	0	100		
	10	0	100		
50	1	0	100	90	-6
	2	0	100		
	3	0	100		
	4	0	100		
	5	1	50		
	6	0	100		
	7	1	50		
	8	0	100		
	9	0	100		
	10	0	100		
130	1	0	100	90	-6
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
	6	1	50		
	7	0	100		
	8	0	100		
	9	0	100		
	10	1	50		
320	1	1	50	25 ^c	71
	2	1	50		
	3	2	0		
	4	2	0		
	5	1	50		
	6	2	0		
	7	2	0		
	8	1	50		
	9	1	50		
	10	2	0		

^a Percent inhibition relative to the pooled control.

^b NA = Not Applicable.

^c Significantly reduced compared to the pooled control, based on Bonferroni's Test.

Table 22. Shoot dry weight of tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
Control	1	0.8202	0.5348	1.1461	0.2911	NA ^d
	2	0.8544	0.0110			
	3	1.4055	NA			
	4	1.3666	0.3344			
	5	1.2430	0.5051			
	6	0.8059	0.3057			
	7	1.6642	NA			
	8	1.0100	0.1051			
	9	0.9982	0.1627			
	10	1.2930	0.4622			
Solvent Control	1	1.0957	0.4067	1.1154	0.3066	NA
	2	1.1212	NA			
	3	1.0004	0.0744			
	4	0.9219	0.1983			
	5	0.7277	0.9747			
	6	1.7374	NA			
	7	1.0817	0.1563			
	8	0.9965	0.0716			
	9	0.9095	NA			
	10	1.5625	NA			
Pooled Control 4.0	1	0.9179	0.2423	1.1308 1.0774	0.2914 0.3001	NA 5
	2	1.4858	NA			
	3	1.3443	NA			
	4	1.1798	0.3246			
	5	0.8981	0.4513			
	6	0.7689	0.8900			
	7	0.8433	0.1638			
	8	0.7947	0.8012			
	9	1.5867	0.7528			
	10	0.9548	NA			
10	1	0.8787	0.2399	0.9528	0.0994	16
	2	0.9287	0.4328			
	3	1.0453	0.0530			
	4	1.1462	NA			
	5	1.0007	0.1634			
	6	NA	NA			
	7	0.9701	0.4747			
	8	0.8388	0.1770			
	9	0.8530	0.0128			
	10	0.9140	0.3657			

^a SD = Standard Deviation.

^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.

^c Percent inhibition relative to the pooled control.

^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

^e Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 22. (continued) Shoot dry weight of tomato (*Lycopersicon esculentum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	Inhibition (%) ^c
20	1	0.8528	NA	0.8137	0.1208	28
	2	0.9175	0.0400			
	3	0.6787	0.2734			
	4	0.7082	0.2329			
	5	0.8494	0.0495			
	6	1.0783	0.0223			
	7	0.7039	0.0378			
	8	0.8053	0.2370			
	9	0.7351	0.2472			
	10	0.8082	0.0343			
50	1	0.6336	0.0211	0.6790 ^e	0.1390	40
	2	0.6536	0.0063			
	3	0.6061	0.1928			
	4	0.6525	0.0313			
	5	1.0211	NA			
	6	0.5924	0.2635			
	7	0.7874	NA			
	8	0.5130	0.0595			
	9	0.6868	0.1346			
	10	0.6432	0.0723			
130	1	0.0795	0.0818	0.1568 ^e	0.0753	86
	2	0.2097	0.1390			
	3	0.0544	0.0292			
	4	0.2398	0.0141			
	5	0.1370	0.1763			
	6	0.2605	NA			
	7	0.1538	0.0234			
	8	0.2294	0.0535			
	9	0.1371	0.1009			
	10	0.0671	NA			
320	1	0.0003	NA	0.0206 ^e	0.0288	98
	2	0.0028	NA			
	3	NA	NA			
	4	NA	NA			
	5	0.0670	NA			
	6	NA	NA			
	7	NA	NA			
	8	0.0024	NA			
	9	0.0305	NA			
	10	NA	NA			

^a SD = Standard Deviation.

^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.

^c Percent inhibition relative to the pooled control.

^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.

^e Significantly reduced compared to the pooled control, based on Kruskal-Wallis' Test.

Table 23. **The EC25, EC50, NOEC and LOEC values for bisphenol A calculated from the 21-day toxicity test results (percent emergence and dry shoot weight) with tomato (*Lycopersicon esculentum*).**

Percent Emergence	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	190	260	320	130
95% confidence limits:	160 – 210	230 – 300		
Dry Shoot Weight	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	19	67	50	20
95% confidence limits:	9.8 – 32	52 - 79		

Table 24. Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for wheat (*Triticum aestivum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Condition at Test Termination					
	Plant Number	1	2	Replicate 3	4	5
Control	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	H	H	H	H	H
Solvent Control	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	Ne	H	H	H	H
3.8	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	Ne	H	H	H	H
9.4	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	H	H	H	H	H

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 24. (continued) Observations of morphological abnormalities, plant condition (at test termination) and mortalities (throughout the test period) for wheat (*Triticum aestivum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Plant Condition at Test Termination					
	Plant Number	1	2	Replicate		
				3	4	5
20	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	H	H	H	Ne	H
47	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	H	H	H	H	H
120	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	H	H	Ne	H	H
300	1	H	H	H	H	H
	2	H	H	H	H	H
	3	H	H	H	H	H
	4	H	H	H	H	H
	5	H	H	H	H	H
	6	H	H	H	H	H
	7	H	H	H	H	H
	8	H	H	Ne	H	H

H = Healthy

Ne = Non-emerged

N = Necrotic

Dp = Dead plant

Table 25. Percent emergence of wheat (*Triticum aestivum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Replicate	Number Non-Emerged	Day 21 % Emerged	Treatment % Emerged Mean	Inhibition (%) ^a
Control	1	0	100	100	NA ^b
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
Solvent Control	1	1	88	98	NA
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
Pooled Control				99	NA
3.8	1	1	88	98	1
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
9.4	1	0	100	100	-1
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
20	1	0	100	98	1
	2	0	100		
	3	0	100		
	4	1	88		
	5	0	100		
47	1	0	100	100	-1
	2	0	100		
	3	0	100		
	4	0	100		
	5	0	100		
120	1	0	100	98	1
	2	0	100		
	3	1	88		
	4	0	100		
	5	0	100		
300	1	0	100	98	1
	2	0	100		
	3	1	88		
	4	0	100		
	5	0	100		

^a Percent inhibition relative to the pooled control.^b NA = Not Applicable.

Table 26. Shoot dry weight of wheat (*Triticum aestivum*) plants exposed to bisphenol A during the seedling emergence and growth test.

Nominal Concentration (mg a.i./kg)	Day-21 Shoot Dry Weight (g)					Inhibition (%) ^c
	Replicate	Replicate Mean	SD ^a	Treatment Mean ^b	SD	
Control	1	0.1715	0.0406	0.1900	0.0192	NA ^d
	2	0.1768	0.0325			
	3	0.2195	0.0341			
	4	0.1974	0.0319			
	5	0.1846	0.0329			
Solvent Control	1	0.1794	0.0538	0.1609	0.0178	NA
	2	0.1589	0.0203			
	3	0.1678	0.0213			
	4	0.1319	0.0285			
	5	0.1666	0.0376			
3.8	1	0.2044	0.0239	0.1685	0.0206	-5
	2	0.1568	0.0232			
	3	0.1532	0.0215			
	4	0.1652	0.0444			
	5	0.1626	0.0211			
9.4	1	0.1585	0.0189	0.1541	0.0046	4
	2	0.1595	0.0254			
	3	0.1522	0.0161			
	4	0.1493	0.0419			
	5	0.1512	0.0206			
20	1	0.1975	0.0548	0.1858	0.0085	-15
	2	0.1852	0.0190			
	3	0.1823	0.0352			
	4	0.1746	0.0195			
	5	0.1895	0.0163			
47	1	0.1821	0.0372	0.1678	0.0161	-4
	2	0.1820	0.0485			
	3	0.1710	0.0198			
	4	0.1443	0.0559			
	5	0.1594	0.0295			
120	1	0.1033	0.0292	0.1230 ^d	0.0176	24
	2	0.1052	0.0191			
	3	0.1404	0.0434			
	4	0.1364	0.0386			
	5	0.1295	0.0387			
300	1	0.0364	0.0382	0.0297 ^d	0.0101	82
	2	0.0300	0.0299			
	3	0.0342	0.0260			
	4	0.0121	0.0070			
	5	0.0359	0.0265			

^a SD = Standard Deviation.^b Treatment results were calculated from the unrounded raw data and not the rounded replicate values presented in this table.^c Percent inhibition relative to the solvent control.^d NA = Not Applicable. Relative to a standard deviation, if two plants or less were present a standard deviation could not be calculated.^e Significantly reduced compared to the solvent control, based on Dunnett's Test.

Table 27. The EC25, EC50, NOEC and LOEC values for bisphenol A calculated from the 21-day toxicity test results (percent emergence and dry shoot weight) with wheat (*Triticum aestivum*).

Percent Emergence	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	>300	>300	>300	300
95% confidence limits:	NA ^a	NA		

Dry Shoot Weight	EC25	EC50	LOEC	NOEC
EC value (mg a.i./kg):	120	200	120	47
95% confidence limits:	98 – 140	180 - 210		

^a NA = Not Applicable. EC25 and EC50 values were empirically estimated, therefore, 95% confidence limits could not be calculated.

Table 28. Summary of the EC25, EC50, NOEC and LOEC values for bisphenol A calculated from the 21-day toxicity test results (percent emergence and dry shoot weight).

Species	Percent Emergence Results (as mg a.i./kg) ^a			
	EC25 ^b	EC50 ^b	LOEC	NOEC
Cabbage	130 (83 – 180)	190 (120 – 230)	320	130
Corn	>320 NA ^c	>320 NA ^c	>320	320
Oat	>800 NA ^c	>800 NA ^c	>800	800
Soybean	650 (370 – 800)	>800 NA ^c	>800	800
Tomato	190 (160 – 210)	260 (230 – 300)	320	130
Wheat	>300 NA ^c	>300 NA ^c	>300	300
Species	Dry Shoot Weight Results (as mg a.i./kg) ^a			
	EC25 ^b	EC50 ^b	LOEC	NOEC
Cabbage	82 (52 – 120)	>130 NA ^c	130	50
Corn	83 (14 – 180)	160 (80 – 280)	320	130
Oat	69 (57 – 81)	100 (87 – 130)	120	47
Soybean	220 (72 – 360)	460 (370 – 520)	800	320
Tomato	19 (9.8 – 32)	67 (52 – 79)	50	20
Wheat	120 (98 – 140)	200 (180 – 210)	120	47

^a Results are based on nominal concentrations.

^b 95% confidence limits are presented in parenthesis.

^c NA = Not Applicable. EC25 and EC50 values were empirically estimated, therefore, 95% confidence limits could not be calculated.

APPENDIX 1 - STUDY PROTOCOL

**PROTOCOL TITLE: Seedling Emergence and Seedling Growth Test Following OECD
Draft Guideline #208**

TO BE COMPLETED BY THE STUDY SPONSOR:	
Study Sponsor:	American Chemistry Council
Address:	1300 Wilson Blvd Arlington, VA 22209 Phone: 919-549-2236
Study Monitor:	Tilghman Hall E-mail: tilghman.hall@bayercropscience.com
Study Sponsor Representative:	Steven Hentges E-mail: steve_hentges@plastics.org
Sponsor Protocol/Project No.:	
Test Substance Name(s): Bisphenol A	
Purity: 99.62%	Batch or Lot #: B0070138
Analytical Standard: Bisphenol A	
Purity: 99.62%	Batch or Lot #: B0070138
Additional Comments and Modifications:	
Monitor	
Sponsor Representative Approval:	<i>Tilghman Hall</i> Date: 4/27/07
Representative	
Study Monitor Approval:	<i>S. Hentges</i> Date: 4/30/07

TO BE COMPLETED BY SPRINGBORN SMITHERS LABORATORIES BEFORE EXPERIMENT INITIATION:

Testing Facility: Springborn Smithers Laboratories 790 Main Street, Wareham, MA 02571-1037

Study Director: James Hoberg

Study No.: 13761.6124

Test Concentration: Provided in Section 2.1.2 of this protocol

Proposed Experimental Dates:

(Start) 25 April 2007

(Termination) 25 May 2007

James R. Hoberg
Study Director Signature*30 April 2007*
Study Initiation Date

* To be provided by protocol amendment, if applicable.

Seedling Emergence and Seedling Growth Test Following OECD Draft Guideline #208**1.0 OBJECTIVE**

The purpose of this study is to determine the effects (e.g., EC₂₅ and EC₅₀ values) of bisphenol A on seedling emergence and shoot dry weight biomass of six plant species. The number of emerged seedlings will be recorded 7, 14 and 21 days after 50% of the control seedlings have emerged. Emergence is defined as the appearance of plant tissue above the surface of the support substrate. Observations will be recorded weekly for mortality and visual phytotoxicity (chlorosis, necrosis, etc.). At test termination, the number of emerged seedling, dry shoot weight and visual phytotoxicity will be recorded.

Replicate test and control pots will be randomly placed within blocks in the greenhouse. The means and standard deviations will be calculated for control and treatment replicate measurements.

If a concentration response is observed, EC₂₅ and EC₅₀ values will be determined for percent emergence and dry shoot weight. If less than 25% response is observed, the EC values will be stated as greater than the highest concentration tested.

2.0 MATERIALS AND METHODS**2.1 Chemical System****2.1.1 Test Substance**

Upon arrival at Springborn Smithers Laboratories, the test and reference substance(s) will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test substance will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

The sample will be given a unique sample ID number and stored under the conditions specified by the Sponsor or manufacturer. The following information should be provided by the Study Sponsor, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

2.1.2 Test Substance Concentration Selection

Definitive test concentrations have been selected in consultation with the Study Sponsor and based on previous testing at 150 and 1000 mg a.i./kg for each test species. The nominal test concentrations for this study are presented below:

Cabbage: 20, 50, 130, 320 and 800 mg a.i./kg

Corn: 4.0, 10, 20, 50, 130 and 320 mg a.i./kg

Oats: 10, 20, 50, 130, 320 and 800 mg a.i./kg

Soybean: 20, 50, 130, 320 and 800 mg a.i./kg

Tomato: 4.0, 10, 20, 50, 130 and 320 mg a.i./kg

Wheat: 4.0, 10, 20, 50, 130, 320 mg a.i./kg

2.1.3 Stock Solutions and Exposure Soil Preparation

A Chemical Usage Log will be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test substance is used. A primary stock solution will be prepared by dissolving the appropriate amount of test substance in acetone. Secondary stock solutions will be prepared in acetone from dilutions of the primary stock solution.

A separate batch (6 or 12 kg) of soil will be dosed for each treatment and each species. For the tests with cabbage, corn, soybean, oats and tomato, 50 mL of the appropriate stock solution will be applied to 0.50 kg silica sand and the sand will be placed in a fume hood until the solvent evaporates. The treated sand will then be dispersed into 11.5 kg (dry weight) of sandy loam soil and mixed with a Hobart mixer for ten minutes to provide the desired nominal concentrations. For the test with wheat, a total of 5.5 kg of sandy loam soil will be treated as noted above.

2.1.4 Solvent Control

Since acetone will be used to solubilize the test substance, a solvent control will be included in the study design. The solvent control will consist of 50 mL of acetone applied to 0.50 kg of silica sand, the acetone will be evaporated from the sand and the sand will be blended in sandy loam soil as mentioned above. The solvent control soil will be prepared first, followed by the treatments.

2.1.5 Control

A negative control will be included and will consist of untreated sandy loam soil

2.2 Test System

2.2.1 Species

The use of six test species helps to ensure that variations in seedling response to the test substance are detected. Recommended test species (OECD, 2003) are the following:

Monocotyledon:

Avena sativa – oats
Triticum aestivum – wheat
Zea mays – corn

Dicotyledon:

Brassica oleracea – cabbage
Glycine max – soybean
Lycopersicon esculentum – tomato

2.2.2 Justification of Test System

Selection of plant species for testing is based on several criteria including germination time, seedling size, sensitivity to chemical challenges and existing data base in toxicology studies.

2.2.3 Source

Seeds used for testing will not have been pretreated with fungicides or insecticides to avoid potential interactions with the test substance. The seed species, variety, source, lot number, and the germination percentage will be documented. The seeds will be purchased from a commercial supplier whose identity will be documented in the data and in the final report.

2.3 Physical System**2.3.1 Support Medium**

A heat-sterilized, natural sandy loam will be used as the support medium. The organic carbon content of the soil will be <1.5%. Soil moisture at the time of dosing will be approximately 1%. A representative sample of the sandy loam has been analyzed for the absence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, MA. Characterization of other physical parameters (i.e., particle size, cation exchange capacity) of the support medium has also been performed by Agvise Laboratories, Northwoods, ND. Soil characterization will be provided in the final report. The support medium (approximately 1.2 kg, dry weight) will be contained within a polypropylene pot (top diameter = 14 cm, bottom diameter = 12 cm, height 12 cm, depth of medium = 10 cm).

2.3.2 Replication and Control of Bias

The following table presents species, replication and number of seeds exposed.

Species	Number of Replicates	Number of Seeds/replicate
Cabbage	10	4
Wheat	5	8
Corn	10	2
Soybean	10	2
Oats	10	8
Tomato	10	2

Treatment and control replicates will be positioned in a randomized block format based on computer-generated random numbers within the greenhouse.

2.4 Test Procedures

2.4.1 Seedling Exposure Method

All pots will be labeled with the study number, test species, test concentration and replicate. Seeds will be planted approximately 1 to 2 cm below the surface of the support substrate. The exposure soil will be prepared as described in Section 2.1.3.

2.4.2 Irrigation

The plants will be irrigated using a commercially prepared water-soluble fertilizer dissolved in well water. The type of fertilizer used will be identified in the raw data and final report. The fertilizer will be provided to each replicate pot by subirrigation at a rate of approximately 100 mL twice weekly. All subsequent watering will be with well water on an as needed basis. Each addition of dilute fertilizer and well will be recorded in the raw data.

2.4.3 Environmental Conditions

Light intensity, relative humidity and temperature will be monitored and recorded daily throughout the test period. Whenever natural light falls below 800 foot-candles (8600 lux), sodium vapor lights will turn on until natural light is restored or until the end of the light period (16 hours light: and 8 hours dark). The greenhouse temperature is generally expected to be 15 to 35 °C. Heating and cooling will cycle as required to maintain optimum growth.

2.4.4 Seedling Observations

Each control replicate will be observed four days after the exposure is initiated to determine the number of seedlings that have emerged. If ≥50% emergence is not observed, the control replicates will be observed daily until this criterion is met. Seven, 14 and 21 days after ≥50% emergence is determined in the control, the number of emerged plants will be recorded in all pots. Additionally, all plants will be observed at these weekly intervals for visual phytotoxicity (e.g., morphological abnormalities, chlorosis, necrosis) and mortality. The test will be terminated on day 21 post ≥50% emergence. At test termination, the above ground portion of the plants (shoots) will be harvested. Shoots will be placed in pre-weighed containers (e.g., tins, bags), dried at least three days at 70 ± 5 °C and weighed on an analytical balance to determine individual shoot dry weight.

Observation of morphological abnormalities will be evaluated with a rating scale based on the percentage of the plants exhibiting the abnormality. The rating scale will be from 0 to 100, where 0 indicates no injury or abnormalities and 100 indicates a dead plant.

2.5 Analytical Methodology

A sample of each stock solution and acetone used to treat the soil will be collected on day 0 and analyzed for bisphenol A concentration by HPLC-UV methodology. Three quality control (QC) samples will be prepared, stored if necessary, and analyzed with the set of study samples. Results of these analyses will indicate the accuracy of the analytical method for measuring test substance concentrations. The analytical method will be verified by Springborn Smithers Laboratories prior to test initiation.

3.0 DATA ANALYSIS

Test data will be presented in tabular format that includes observation date, percent emergence and dry shoot weight. The means and standard deviations for control(s) and treatment replicate measurements will be calculated.

The control and solvent control data will be compared using a two-tailed t Test. If the data are similar, the control and solvent control values will be pooled for further analysis with the treatment data. If a significant difference is detected between the control and solvent control data, the treatment data will be compared to the solvent control data.

Mean percent emergence and mean shoot dry weight of the treated plants will be calculated as a percentage relative to the appropriate control data (e.g., percent inhibition). Additionally, the EC_{25} and EC_{50} values and 95% confidence intervals will be calculated for seedling emergence and shoot dry weight using the IC_p method (U.S. EPA, Norberg-King) in the software package TOXSTAT version 3.5 (Gulley et. al., 1996).

4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

5.0 REPORTING

The raw data and final draft of the report will be reviewed by the Quality Assurance Unit and the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. A single copy of the draft report will initially be submitted to the Study Sponsor for review. Upon acceptance by the Sponsor, a copy of the final report will be submitted. All reports will include, but will not be limited to, the following information:

- Springborn Smithers Laboratories report and project numbers and Sponsor study numbers (if any).
- Laboratory and site, the dates of testing and personnel involved in the study, i.e., Program Coordinator (if applicable), Study Director, Principal Investigator.

- Identification of the test substance which may include chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, water solubility, vapor pressure, degree of purity of test article (percent test chemical) (Sponsor supplied, if available).
- Information about the test plants: species and variety used, seed source (packager or supplier), seed lot, and germination percentage.
- Description of the test method or attached literature reference describing the method used.
- Conditions of testing:
 - a. Carriers, emulsifiers, solvents, and/or additives used and their concentrations.
 - b. Mean test temperature (\pm standard deviation) and range throughout the test period.
 - c. Photoperiod if conducted under light/dark conditions and mean light intensity of the test area.
 - d. Relative humidity range throughout the test.
 - e. Method of test chemical introduction and concentrations.
 - f. Source and description of water used to prepare the water-soluble fertilizer.
 - g. Number of replicates per concentration or control.
 - h. Characterization of the support medium (e.g., percent-organic matter, pH).
 - i. Method of assignment and positioning of seeds/seedlings.
- Number and percentage of seedlings that showed any adverse effect in the controls and treatments at the conclusion of the test.
- A description of the statistical procedures used, EC_{25} and EC_{50} values, and their 95% confidence intervals, for seedling emergence and shoot dry weight data.
- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.
- Location of raw data and report.

6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any. If necessary, amendments other than the one providing the information required by page one of this protocol, may initially be verbally authorized, followed by Springborn Smithers' written documentation. In such cases, the effective date of the amendment will be the date of verbal authorization.

7.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records and reports will comply with the Organization of Economic Co-operation and Development's (OECD) Good Laboratory Practices as set forth under the OECD Guidelines for the Testing of Chemicals.

8.0 REFERENCES

- Gulley, D.D., Boettler, A.M. and Bergman, H.L. 1996 Toxstat Release 3.5. University of Wyoming, Laramie, Wyoming.
- OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris, France. 41 pp.
- OECD, 2003. OECD Guidelines for the Testing of Chemicals, Proposal for Updating Guideline 208. September 2003.
- U.S. EPA. 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants. PB83-153940, U. S. Environmental Protection Agency, Washington, DC.
- U.S. EPA. 1986. Hazard Evaluation Division. Standard Evaluation Procedure. Non-Target Plants: Seed Germination/Seedling Emergence/ Vegetative Vigor. EPA 540/9-86-132. U.S. EPA Washington, D.C.
- U.S. EPA. 1994. Pesticide Reregistration Rejection Rate Analysis: Ecological Effects. EPA 738-R-94-035, U.S. Environmental Protection Agency, Washington, DC.
- Zar, J.H. 1984. Biostatistical Analysis, 2 ed. Prentice Hall, Inc.: Englewood Cliffs, NJ, 718 pp.

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Massachusetts Research Center

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PROTOCOL AMENDMENT

Amendment No.: 1
Effective Date: 17 June 2007
Protocol Title: Seedling Emergence and Seedling Growth Test Following OECD Draft Guideline #208
Protocol Number: 041207/OECD/Emergence and Growth/6 species/BPA
Species: 6 species
Study Sponsor: American Chemistry Council
Test Substance: Bisphenol A
Springborn Study No.: 13761.6124

Amendment:**1. Page 1, Cover Page**

To clarify the corrections in the Study Monitor and Study Representative approvals, they were made at the time of signing by one of these personnel to reflect the correct title of the individuals.

2. Page 5, Section 2.4.2 Irrigation

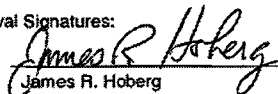
At the Study Sponsor's request, a sample of the well water to be used to irrigate the plants will be collected and analyzed for residual Bisphenol A concentration. The same water source will be used to prepare the water-soluble fertilizer also to be used to irrigate the plants. The analysis will be performed by ABC Laboratories, Columbia, Missouri. Two additional water samples will be collected and held at Springborn Smithers Laboratories as archive samples in the event they are needed for analysis.


3. Page 6, Section 3.0 Data Analysis

The No-Observed-Effect Concentration (NOEC) and Low-Observed-Effect Concentration (LOEC) will also be determined for each biological endpoint (e.g., percent emergence and dry shoot weight). The data will first be checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test ($p \leq 0.01$). If these assumptions are met, either Dunnett's Test or Bonferroni's Test ($p \leq 0.05$) will be used to determine the NOEC and LOEC values. If the assumptions of normality and homogeneity of variance are not met, the NOEC and LOEC values will be determined by Kruskal-Wallis' Test.

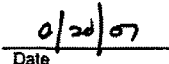
None of the above changes will have a negative impact on the study.

Approval Signatures:


James R. Hoberg
Springborn Study Director


Date 6/26/07


Study Sponsor Representative


Date 6/26/07

Page 1 of 1

Other Locations: 2900 Quakerbush Road, P.O. Box 620 • Snow Camp, North Carolina 27349 • Phone: (336) 376-0141
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PROTOCOL AMENDMENT

Amendment No.: 2
Effective Date: 26 June 2007
Protocol Title: Seedling Emergence and Seedling Growth Test Following OECD Draft Guideline #208
Protocol Number: 041207/OECD/Emergence and Growth/6 species/BPA
Species: 6 species
Study Sponsor: American Chemistry Council
Test Substance: Bisphenol A
Springborn Study No.: 13761.6124

Amendment:

1. Page 3, Section 2.1.2 Test Substance Concentration Selection

The protocol states the nominal test concentrations will be as follows for corn, oat and wheat:

Corn: 4.0, 10, 20, 50, 130 and 320 mg a.i./kg
Oats: 10, 20, 50, 130, 320 and 800 mg a.i./kg
Wheat: 4.0, 10, 20, 50, 130 and 320 mg a.i./kg

Due to a limited amount of stock solution available at the time of dosing, which was a result of collection of the analytical samples, the nominal concentrations for the above species based on actual addition of stock solution were revised to:

Corn: 3.8, 10, 20, 50, 130 and 320 mg a.i./kg
Oats: 9.4, 19, 47, 120 and 300 mg a.i./kg
Wheat: 3.8, 9.4, 20, 47, 120 and 300 mg a.i./kg

The nominal concentrations for the remaining test species were unaffected by the collections of samples for analysis.

None of the above changes will have a negative impact on the study.

Approval Signatures:

James R. Hoberg
Springborn Study Director

8/10/07
Date

Study Sponsor Representative

8-7-07
Date

Page 1 of 1

Other Locations: 2900 Quakenbush Road, P.O. Box 620 • Snow Camp, North Carolina 27349 • Phone: (336) 376-0141
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PROTOCOL AMENDMENT

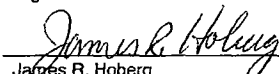
Amendment No.: 3
Effective Date: 6 August 2007
Protocol Title: Seedling Emergence and Seedling Growth Test Following OECD Guideline #208
Protocol Number: 041207/OECD/Emergence and Growth/6 species/BPA
Species: 6 species
Study Sponsor: American Chemistry Council
Test Substance: Bisphenol A
Springborn Study No.: 13761.6124

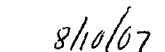
Amendment:**1. Page 1 and 2, Protocol Title**

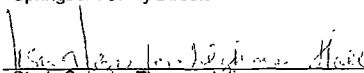
The protocol title is changed to: Seedling Emergence and Seedling Growth Tests Following OECD Guideline 208. The word "draft" was deleted from the title since the guideline was finalized on 19 July 2006 and is no longer a draft guideline.

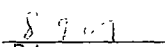
None of the above changes will have a negative impact on the study.

Approval Signatures:


James R. Hoberg
Springborn Study Director


Date


Study Sponsor Representative


Date

Page 1 of 1

Other Locations: 2900 Quakenbush Road, P.O. Box 620 • Snow Camp, North Carolina 27349 • Phone (336) 376-0141
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APPENDIX 2 - CERTIFICATE OF ANALYSIS

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**RTI INTERNATIONAL
COMPOUND ANALYSIS REPORT
BISPHENOL A**

Analysis Date: October 11, 2006
Date of This Report: February 9, 2007

RTI Project No.: 0209257.001
RTI Protocol No.: RTI-675-AN
RTI Notebook No.: 11341 pp.: 50-73

Compound: Bisphenol A
CAS No.: 80-05-7
Formula: $C_{15}H_{16}O_2$
Formula Weight: 228.28
Vendor: Acros Organics
Vendor Lot No.: B0070138
Analytical Sample Log No.: 9176-36-01
Storage Conditions: Room temperature
Appearance: Opaque white granular solid

Purity Determination

HPLC (UV at 210 nm): 99.62% of total integrated area

Component	Retention Time (min)	% of Total area
impurity A	5.4	< 0.01
impurity B	5.8	< 0.01
impurity C	6.8	< 0.01
Bisphenol A	8.0	99.62
impurity D	10.1	0.12
impurity E	13.6	0.01
impurity F	14.7	0.01
impurity G	22.0	0.01
impurity H	24.0	0.01
impurity I	25.2	< 0.01
impurity J	27.7	0.02
impurity K	28.9	0.01
impurity L	34.2	0.12
impurity M	35.9	0.08

Comment: Technical questions about this compound analysis should be directed to
Mr. Stephen D. Cooper at (919) 541-6595

Verified by: *H. E. Amato* Date: 2/9/2007

Approved by: *S. D. Cooper* Date: 2/9/2007

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